10/642,289

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=> d his

     (FILE 'HOME' ENTERED AT 14:21:23 ON 21 DEC 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:24:12 ON 21 DEC 2005
L1         23924 S SPHINGOSINE
L2          2257 S L1(A)KINASE?
L3           113 S HUMAN (A)L2
L4       7440422 S CLON? OR EXPRESS? OR RECOMBINANT
L5            88 S L3 AND L4
L6            44 DUP REM L5 (44 DUPLICATES REMOVED)
                 E PITSON S M/AU
L7            95 S E3
                 E WATTENBERG B W/AU
L8           118 S E3
                 E XIA P/AU
L9           473 S E3
                 E GAMBLE J R/AU
L10          361 S E3
                 E VADAS M A/AU
L11         1308 S E3-E10
L12         1963 S L7 OR L8 OR L9 OR L10 OR L11
L13          101 S L2 AND L12
L14           77 S HUMAN AND L13
L15           24 DUP REM L14 (53 DUPLICATES REMOVED)
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NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	SEP 09	ACD predicted properties enhanced in REGISTRY/ZREGISTRY
NEWS	4	OCT 03	MATHDI removed from STN
NEWS	5	OCT 04	CA/CAplus-Canadian Intellectual Property Office (CIPO) added to core patent offices
NEWS	6	OCT 13	New CAS Information Use Policies Effective October 17, 2005
NEWS	7	OCT 17	STN(R) AnaVist(TM), Version 1.01, allows the export/download of CAplus documents for use in third-party analysis and visualization tools
NEWS	8	OCT 27	Free KWIC format extended in full-text databases
NEWS	9	OCT 27	DIOGENES content streamlined
NEWS	10	OCT 27	EPFULL enhanced with additional content
NEWS	11	NOV 14	CA/CAplus - Expanded coverage of German academic research
NEWS	12	NOV 30	REGISTRY/ZREGISTRY on STN(R) enhanced with experimental spectral property data
NEWS	13	DEC 05	CASREACT(R) - Over 10 million reactions available
NEWS	14	DEC 14	2006 MeSH terms loaded in MEDLINE/LMEDLINE
NEWS	15	DEC 14	2006 MeSH terms loaded for MEDLINE file segment of TOXCENTER
NEWS	16	DEC 14	CA/CAplus to be enhanced with updated IPC codes
NEWS	17	DEC 16	MARPATprev will be removed from STN on December 31, 2005
NEWS	18	DEC 21	IPC search and display fields enhanced in CA/CAplus with the IPC reform

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NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

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FILE 'HCAPLUS' ENTERED AT 14:24:12 ON 21 DEC 2005
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FILE 'LIFESCI' ENTERED AT 14:24:12 ON 21 DEC 2005
COPYRIGHT (C) 2005 Cambridge Scientific Abstracts (CSA)


=> s sphingosine
L1         23924 SPHINGOSINE

=> s l1(a)kinase?
L2          2257 L1(A) KINASE?

=> s human (a)l2
L3           113 HUMAN (A) L2

=> s clon? or express? or recombinant
L4       7440422 CLON? OR EXPRESS? OR RECOMBINANT

=> s l3 and l4
L5            88 L3 AND L4

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6            44 DUP REM L5 (44 DUPLICATES REMOVED)

=> d 1-44 ibib ab

L6   ANSWER 1 OF 44  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:         2005:647415  HCAPLUS
DOCUMENT NUMBER:          143:438271
TITLE:                    Basal and induced sphingosine kinase 1 activity in
                          A549 carcinoma cells: function in cell survival and
                          IL-1β and TNF-α induced production of
                          inflammatory mediators
AUTHOR(S):                Billich, Andreas; Bornancin, Frederic;
                          Mechtcheriakova, Diana; Natt, Francois; Huesken,
```

Dieter; Baumruker, Thomas
CORPORATE SOURCE: Novartis Institutes for BioMedical Research, Vienna, A-1235, Austria
SOURCE: Cellular Signalling (2005), 17(10), 1203-1217
CODEN: CESIEY; ISSN: 0898-6568
PUBLISHER: Elsevier B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sphingosine-1-phosphate, a lipid mediator produced by sphingosine kinases, regulates diverse cellular processes, ranging from cell growth and survival to effector functions, such as proinflammatory mediator synthesis. Using human A549 epithelial lung carcinoma cells as a model system, the authors observed transient upregulation of sphingosine kinase type 1 (SPHK1) enzyme activity upon stimulation with both TNF-α or IL-1β. This transient activation of SPHK1 was required for cytokine-induced COX-2 transcription and PGE2 production, since not only specific siRNA (abolishing both basal and induced SPHK1 enzyme activity), but also a dominant-neg. SPHK1 mutant (suppressing induced SPHK1 activity only) both reduced COX-2 and PGE2. Furthermore, TNF-α- or IL-1β-induced transcription of selected cytokines, chemokines, and adhesion mols. (IL-6, RANTES, MCP-1, and VCAM-1) was found to require SPHK1 activation. Suppression of SPHK1 activation led to reduction of cytokine-induced IκBα phosphorylation and consequently diminished NFκB activity due to reduced nuclear translocation of RelA (p65), explaining the dependence of inflammatory mediator production on SPHK1 activation. Inhibition of basal SPHK1 activity by N,N-dimethylsphingosine or by downregulation of its **expression** using siRNA induced spontaneous apoptosis in A549 cells, an effect that can be explained through interference with constitutive NFκB activity in this cell type. In contrast, **expression** of the dominant-neg. mutant did not induce apoptosis. These findings demonstrate a role of SPHK1 activation in proinflammatory signaling and of SPHK1 basal activity in survival of A549 lung carcinoma cells.
REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:1038343 HCAPLUS
DOCUMENT NUMBER: 143:419963
TITLE: Immunohistochemical distribution of sphingosine kinase 1 in normal and tumor lung tissue
AUTHOR(S): Johnson, Korey R.; Johnson, Kristy Y.; Crellin, Heather G.; Ogretmen, Besim; Boylan, Alice M.; Harley, Russell A.; Obeid, Lina M.
CORPORATE SOURCE: Department of Medicine, Medical University of South Carolina, Charleston, SC, USA
SOURCE: Journal of Histochemistry and Cytochemistry (2005), 53(9), 1159-1166
CODEN: JHCYAS; ISSN: 0022-1554
PUBLISHER: Histochemical Society, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sphingosine kinase 1 (SK1) is a key enzyme critical to the sphingolipid metabolic pathway responsible for catalyzing the formation of the bioactive lipid sphingosine-1-phosphate. SK1-mediated production of sphingosine-1-phosphate has been shown to stimulate such biol. processes as cell growth, differentiation, migration, angiogenesis, and inhibition of apoptosis. In this study, cell type-specific immunolocalization of SK1 was examined in the bronchus/terminal bronchiole of the lung. Strong immunopos. staining was evident at the apical surface of pseudostratified epithelial cells of the bronchus and underlying smooth muscle cells, submucosal serous glands, immature chondrocytes, type II alveolar cells, foamy macrophages, endothelial cells of blood vessels, and neural bundles. Immunohistochem. screening for SK1 **expression** was performed in

25 samples of normal/tumor patient matched non-small-cell lung cancer tissue and found that 25 of 25 tumor samples (carcinoid [5 samples], squamous [10 samples], and adenocarcinoma tumors [10 samples]), exhibited overwhelmingly pos. immunostaining for SK1 as compared with patient-matched normal tissue. In addition, an approx. 2-fold elevation of SK1 mRNA **expression** was observed in lung cancer tissue vs. normal tissue, as well as in several other solid tumors. Taken together, these findings define the localization of SK1 in lung and provide clues as to how SK1 may play a role in normal lung physiol. and the pathophysiol. of lung cancer.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 44 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:431291 BIOSIS
DOCUMENT NUMBER: PREV200400435848
TITLE: Mammalian sphingosine kinase type 2 isoforms, **cloning**, **expression** and methods of use thereof.
AUTHOR(S): Spiegel, Sarah [Inventor, Reprint Author]; Kohama, Takafumi [Inventor]
CORPORATE SOURCE: McLean, VA, USA
ASSIGNEE: Sankyo Company, Ltd., Tokyo, Japan; Georgetown University
PATENT INFORMATION: US 6800470 20041005
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Oct 5 2004) Vol. 1287, No. 1. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Nov 2004
Last Updated on STN: 10 Nov 2004

AB Nucleic acids encoding mouse and **human sphingosine kinase** type 2 isoforms, methods for detecting agents or drugs which inhibit or promote sphingosine activity and therapeutic agents containing peptides or antibodies to peptides encoded by such nucleic acids.

L6 ANSWER 4 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:80714 HCAPLUS
DOCUMENT NUMBER: 140:141434
TITLE: Human protein sequences of protein complexes of cellular networks underlying the development of cancer and other diseases
INVENTOR(S): Merino, Alejandro; Bouwmeester, Tewis; Bauer, Andreas; Drewes, Gerard; Marzioch, Martina; Kruse, Ulrich; Superti-Furga, Giulio; Eberhard, Dirk; Ruffner, Heinz; Hobson, Scott; Helftenbein, Gerd; Cruciat, Cristina
PATENT ASSIGNEE(S): Cellzome Ag, Germany; et al.
SOURCE: PCT Int. Appl., 810 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004009622	A2	20040129	WO 2003-EP7835	20030718
WO 2004009622	A3	20040910		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,

LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM,
PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:

EP 2002-16109	A	20020719
EP 2002-16111	A	20020719
EP 2002-16123	A	20020719
EP 2002-16128	A	20020719
EP 2002-16427	A	20020722

AB The present invention relates to protein complexes involved in cellular processes which have been shown to be critical for the development of various forms of cancer, component proteins of the said complexes, fragments and derivs. of the component proteins, and antibodies specific to the complexes. The present invention also relates to methods for use of the complexes and their interacting proteins in, inter alia, screening, diagnosis, and therapy, as well as to methods of preparing the complexes.

L6 ANSWER 5 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:533806 HCAPLUS
DOCUMENT NUMBER: 141:84623
TITLE: Use of yeast DPL1, LCB4, and YSR2 mutants **expressing** heterologous sphingolipid pathway enzyme gene in screening for modulators of sphingolipid metabolism and/or signaling
INVENTOR(S): Saba, Julie D.
PATENT ASSIGNEE(S): Children's Hospital and Research Institute at Oakland, USA
SOURCE: U.S. Pat. Appl. Publ., 101 pp., Cont.-in-part of U.S. Ser. No. 348,052.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004126834	A1	20040701	US 2003-622011	20030716
US 2003175939	A1	20030918	US 2002-53510	20020117
US 6830881	B2	20041214		
US 2003219782	A1	20031127	US 2003-348052	20030117

PRIORITY APPLN. INFO.:

US 2002-349582P	P	20020117
US 2002-53510	A2	20020117
US 2003-348052	A2	20030117
US 1997-939309	A2	19970929
US 1999-356643	A2	19990719

AB A method for screening for agents that modulate sphingolipid metabolism and/or signaling pathways comprises culturing of yeast mutants in sphingosine-1-phosphate lyase gene DPL1, sphingosine kinase gene LCB4, and/or sphingosine-1-phosphate phosphatase gene YSR2 which **express** a nonendogenous sphingolipid pathway enzyme gene (such as human SPHK1) in presence of sphingosine and test compound Increased yeast growth in the presence of a test compound indicates the presence of a inhibitor of sphingolipid metabolism Thus, significant accumulation of phosphorylated sphingolipids in S. cerevisiae caused cell death. Yeast with defects in sphingolipid metabolism **expressing human sphingosine kinase** could therefore survive in the presence of inhibitors of the human enzyme.

L6 ANSWER 6 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:1086668 HCAPLUS

DOCUMENT NUMBER: 142:49599
TITLE: Sphingosine Kinase 1 (SPHK1) Is Induced by Transforming Growth Factor-β and Mediates TIMP-1 Up-regulation
AUTHOR(S): Yamanaka, Masayoshi; Shegogue, Daniel; Pei, Heuping; Bu, Shizhong; Bielawska, Alicja; Bielawski, Jacek; Pettus, Benjamin; Hannun, Yusuf A.; Obeid, Lina; Trojanowska, Maria
CORPORATE SOURCE: Division of Rheumatology and Immunology and the Department of Biochemistry and Molecular Biology, Medical University of South Carolina, and the Division of General Internal Medicine, Ralph H. Johnson Veterans Affairs Hospital, Charleston, SC, 29425, USA
SOURCE: Journal of Biological Chemistry (2004), 279(52), 53994-54001
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Transforming growth factor-β (TGF-β) signaling plays a pivotal role in extracellular matrix deposition by stimulating collagen production and other extracellular matrix proteins and by inhibiting matrix degradation The present study was undertaken to define the role of sphingosine kinase (SphK) in TGF-β signaling. TGF-β markedly up-regulated SphK1 mRNA and protein amts. and caused a prolonged increase in SphK activity in dermal fibroblasts. Concomitantly, TGF-β reduced sphingosine-1-phosphate phosphatase activity. Consistent with the changes in enzyme activity, corresponding changes in sphingolipid levels were observed such that sphingosine 1-phosphate (S1P) was increased (.apprx.2-fold), whereas sphingosine and ceramide were reduced after 24 h of TGF-β treatment. Given the relatively early induction of SphK gene **expression** in response to TGF-β, we examined whether SphK1 may be involved in the regulation of TGF-β-inducible genes that exhibit compatible kinetics, e.g. tissue inhibitor of metalloproteinase-1 (TIMP-1). We demonstrate that decreasing SphK1 **expression** by small interfering RNA (siRNA) blocked TGF-β-mediated up-regulation of TIMP-1 protein suggesting that up-regulation of SphK1 contributes to the induction of TIMP-1 in response to TGF-β. The role of SphK1 as a pos. regulator of TIMP-1 gene **expression** was further corroborated by using ectopically **expressed** SphK1 in the absence of TGF-β. Adenovirally **expressed** SphK1 led to a 2-fold increase of endogenous S1P and to increased TIMP-1 mRNA and protein production In addition, ectopic SphK1 and TGF-β cooperated in TIMP-1 up-regulation. Mechanistically, expts. utilizing TIMP-1 promoter constructs demonstrated that the action of SphK1 on the TIMP-1 promoter is through the AP1-response element, consistent with the SphK1-mediated up-regulation of phospho-c-Jun levels, a key component of AP1. Together, these expts. demonstrate that SphK/S1P are important components of the TGF-β signaling pathway involved in up-regulation of the TIMP-1 gene.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 7 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:912420 HCAPLUS
DOCUMENT NUMBER: 141:393902
TITLE: Sphingosine kinase-1 mediates TNF-α-induced MCP-1 gene **expression** in endothelial cells: Upregulation by oscillatory flow
AUTHOR(S): Chen, Xi-Lin; Grey, Janice Y.; Thomas, Suzanne; Qiu, Fei-Hua; Medford, Russell M.; Wasserman, Martin A.; Kunsch, Charles
CORPORATE SOURCE: Discovery Research, AtheroGenics, Alpharetta, GA, 30004, USA

SOURCE: American Journal of Physiology (2004), 287(4, Pt. 2), H1452-H1458
CODEN: AJPHAP; ISSN: 0002-9513
PUBLISHER: American Physiological Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Atherosclerosis is a focal inflammatory disease and preferentially occurs in areas of low fluid shear stress and oscillatory flow, whereas the risk of atherosclerosis is decreased in regions of high fluid shear stress and steady laminar flow. Sphingosine kinase-1 (SphK1) catalyzes the conversion of sphingosine to sphingosine-1 phosphate (S1P), a sphingolipid metabolite that plays important roles in angiogenesis, inflammation, and cell growth. In the present study, we demonstrated that exposure of human aortic endothelial cells to oscillatory flow (shear stress, ±5 dyn/cm2 for 48 h) resulted in a marked increase in SphK1 mRNA levels compared with endothelial cells kept in static culture. In contrast, laminar flow (shear stress, 20 dyn/cm2 for 48 h) decreased SphK1 mRNA levels. We further investigated the role of SphK1 in TNF-α-induced **expression** of inflammatory genes, such as monocyte chemoattractant protein-1 (MCP-1) and VCAM-1 by using small interfering RNA (siRNA) specifically for SphK1. Treatment of endothelial cells with SphK1 siRNA suppressed TNF-α-induced increase in MCP-1 mRNA levels, MCP-1 protein secretion, and activation of p38 MAPK. SphK1 siRNA also inhibited TNF-α-induced cell surface **expression** of VCAM-1, but not ICAM-1, protein. Exposure of endothelial cells to S1P led to an increase in MCP-1 protein secretion and MCP-1 mRNA levels and activation of NF-κB-mediated transcriptional activity. Treatment of endothelial cells with the p38 MAPK inhibitor SB-203580 suppressed S1P-induced MCP-1 protein secretion. These data suggest that SphK1 mediates TNF-α-induced MCP-1 gene **expression** through a p38 MAPK-dependent pathway and may participate in oscillatory flow-mediated proinflammatory signaling pathway in the vasculature.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 8 OF 44 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:44696 SCISEARCH
THE GENUINE ARTICLE: 881YT
TITLE: Identification of genetic and epigenetic similarities of SPHK1/Sphk1 in mammals
AUTHOR: Imamura T; Miyauchi-Senda N; Tanaka A; Shiota K (Reprint)
CORPORATE SOURCE: Univ Tokyo, Lab Cellular Biochem, Bunkyo Ku, 1-1-1 Yayoi, Tokyo 1138657, Japan (Reprint); Univ Tokyo, Lab Cellular Biochem, Bunkyo Ku, Tokyo 1138657, Japan
COUNTRY OF AUTHOR: Japan
SOURCE: JOURNAL OF VETERINARY MEDICAL SCIENCE, (NOV 2004) Vol. 66, No. 11, pp. 1387-1393.
ISSN: 0916-7250.
PUBLISHER: JAPAN SOC VET SCI, UNIV TOKYO, 1-1-1 YAYOI, BUNKYO-KU, TOKYO, 103, JAPAN.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 37
ENTRY DATE: Entered STN: 20 Jan 2005
Last Updated on STN: 20 Jan 2005

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In normal tissues, methylation of CpG islands is generally accepted to be limited to the inactive X-chromosome and imprinting clusters. Gene Sphk1 has shown complex organization, indicated by multiple alternative splicing and tissue-dependent DNA methylation within the limited area (T-DMR) of the CpG island in the rat. Comparisons among human, mouse and rat SPHK1/Sphk1 genomic DNA revealed five coding exons and association of a CpG island at the 5' end in common. We also found two novel subtypes,

for a total of eight mRNA subtypes generated through selective usage of untranslated first exons. A 38-bp region at the 5'-end of T-DMR is highly conserved. This restricted area is specifically hypomethylated in the brain. Here, we examine the complex genetic/epigenetic features of the SPHK1/Sphk1 CpG island, and suggest that the T-DMR is the core target for tissue-dependent CpG island methylation.

L6 ANSWER 9 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2004:687468 HCAPLUS
DOCUMENT NUMBER: 141:362282
TITLE: δ-Catenin/NPRAP (neural plakophilin-related armadillo repeat protein) interacts with and activates sphingosine kinase 1
AUTHOR(S): Fujita, Toshitada; Okada, Taro; Hayashi, Shun; Jahangeer, Saleem; Miwa, Noriko; Nakamura, Shunichi
CORPORATE SOURCE: Division of Biochemistry, Department of Molecular and Cellular Biology, Kobe University Graduate School of Medicine, Kobe, 650-0017, Japan
SOURCE: Biochemical Journal (2004), 382(2), 717-723
CODEN: BIJOAK; ISSN: 0264-6021
PUBLISHER: Portland Press Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sphingosine kinase (SPHK) is a key enzyme catalyzing the formation of sphingosine 1-phosphate (SPP), a lipid messenger that is implicated in the regulation of a wide variety of important cellular events acting through intracellular, as well as extracellular, mechanisms. However, the mol. mechanism of intracellular actions of SPP remains unclear. Here, we have identified δ-catenin/NPRAP (neural plakophilin-related armadillo repeat protein) as a potential binding partner for SPHK1 by yeast two-hybrid screening. From co-immunopptn. analyses, the C-terminal portion of δ-catenin/NPRAP containing the seventh to tenth armadillo repeats was found to be required for interaction with SPHK1. Endogenous δ-catenin/NPRAP was co-localized with endogenous SPHK1 and transfected δ-catenin/NPRAP was co-localized with transfected SPHK1 in dissociated rat hippocampal neurons. MDCK (Madin-Darby canine kidney) cells stably **expressing** δ-catenin/NPRAP contained elevated levels of intracellular SPP. In a purified system δ-catenin/NPRAP stimulated SPHK1 in a dose-dependent manner. Furthermore, δ-catenin/NPRAP-induced increased cell motility in MDCK cells was completely inhibited by dimethylsphingosine, a specific inhibitor of SPHK1. These results strongly suggest that at least some of δ-catenin/NPRAP functions, including increased cell motility, are mediated by an SPHK-SPP signaling pathway.
REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 10 OF 44 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1
ACCESSION NUMBER: 2005:202804 BIOSIS
DOCUMENT NUMBER: PREV200500200384
TITLE: **Expression** of the **human sphingosine kinases** (huSPHKs) in the yeast saccharomyces cerevisiae.
AUTHOR(S): Grosz, Gabor [Reprint Author]; Takacs, Laszlo; Feher, Zsigmond
CORPORATE SOURCE: Med and Hlth Sci CtrDept Human Genet, Univ Debrecen, Debrecen, Hungary
SOURCE: Tissue Antigens, (October 2004) Vol. 64, No. 4, pp. 415. print.
Meeting Info.: 1st International Conference on Basic and Clinical Immunogenomics. Budapest, Hungary. October 03-07, 2004. Hungarian Society for Immunology; Foundation of Inflammation Biology Research; Diamond Congress Ltd.

CODEN: TSANA2. ISSN: 0001-2815.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Jun 2005
Last Updated on STN: 1 Jun 2005

L6 ANSWER 11 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2005:644370 HCAPLUS
DOCUMENT NUMBER: 143:437355
TITLE: IL-6 activates sphingosine kinase in the human multiple myeloma cells
AUTHOR(S): Sun, Huiyan; Duan, Haifeng; Jia, Xiangxu; Li, Qingfang; Liu, Yuhe; Wu, Chutse; Wang, Lisheng
CORPORATE SOURCE: Institute of Radiation Medicine, Academy of Military Medical Sciences, Beijing, 100850, Peop. Rep. China
SOURCE: Junshi Yixue Kexueyuan Yuankan (2004), 28(2), 130-132, 135
CODEN: JYKYEL; ISSN: 1000-5501
PUBLISHER: Junshi Yixue Kexueyuan Yuankan Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB The role of sphingosine kinase in the signal transduction of IL-6 was studied in the multiple myeloma cells. RT-PCR was used to identify the **expression** of sphingosine kinase by SKO-007 cells. The cellular sphingosine kinase activity assay was employed to demonstrate the role of sphingosine kinase in IL-6 signal transduction. SKO-007 cells **expressed** sphingosine kinase and the receptors of sphingosine 1-phosphate, endothelial differentiation gene 1, 3, 5. IL-6 activated sphingosine kinase in the human multiple myeloma cells via PI-3K and MAPK pathways. Sphingosine kinase is involved in the IL-6 signal transduction in the human multiple myeloma cells and is likely to be a therapeutic target.

L6 ANSWER 12 OF 44 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2004342307 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15246004
TITLE: An assay for sphingosine kinase activity using biotinylated sphingosine and streptavidin-coated membranes.
AUTHOR: Roberts Jane L; Moretti Paul A B; Darrow Andrew L; Derian Claudia K; Vadas Mathew A; Pitson Stuart M
CORPORATE SOURCE: Hanson Institute, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, SA 5000, Australia.
SOURCE: Analytical biochemistry, (2004 Aug 1) 331 (1) 122-9.
Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200501
ENTRY DATE: Entered STN: 20040713
Last Updated on STN: 20050129
Entered Medline: 20050128
AB Sphingosine kinase catalyses the phosphorylation of sphingosine to generate sphingosine 1-phosphate, a lipid signaling molecule implicated in roles in a diverse range of mammalian cell processes through its action as both a ligand for G-protein-coupled cell-surface receptors and an apparent intracellular second messenger. This paper describes a rapid, sensitive, and reproducible assay for sphingosine kinase activity using biotinylated sphingosine (biotinyl-Sph) as a substrate and capturing the phosphorylated product with streptavidin-coated membranes. We have shown that both **human sphingosine kinase** 1 and 2 (hSK1 and hSK2) can efficiently phosphorylate biotinyl-Sph, with K(m) values similar

to those of sphingosine. The assay utilizing this substrate has high sensitivity for hSK1 and hSK2, with detection limits in the low-femtomole range for both purified **recombinant** enzymes. Importantly, we have also demonstrated the capacity of this assay to measure endogenous sphingosine kinase activity in crude cell extracts and to follow changes in this activity following sphingosine kinase activation. Together, these results demonstrate the potential utility of this assay in both cell-based analysis of sphingosine kinase signaling pathways and high-throughput screens for agents affecting sphingosine kinase activity in vitro.

L6 ANSWER 13 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:335288 HCAPLUS
DOCUMENT NUMBER: 138:349758
TITLE: DNA sequence of promoter for **human sphingosine kinase** 1 and uses
INVENTOR(S): Kohama, Takafumi; Sugiura, Masako
PATENT ASSIGNEE(S): Sankyo Company, Limited, Japan
SOURCE: PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003035871	A1	20030501	WO 2002-JP10882	20021021
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
JP 2003199590	A2	20030715	JP 2002-307956	20021023

PRIORITY APPLN. INFO.: JP 2001-325402 A 20011023
AB This invention provides DNA sequence of promoter for **human sphingosine kinase** 1. The **expression** level of reporter gene was enhanced when the **expression** was regulated under sphingosine kinase 1 promoter. The promoter provided in this invention can be used for diagnosis, treatment and screening the drugs for arteriosclerosis, diabetes, thrombosis, inflammation, immunopathy, allergy, cancer and cancer metastasis.
REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 14 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:301229 HCAPLUS
DOCUMENT NUMBER: 138:316762
TITLE: **Human sphingosine kinase** 3, encoding cDNA, and use in drug screening and diagnosis
INVENTOR(S): Igarashi, Yasuyuki; Kihara, Akio
PATENT ASSIGNEE(S): Hokkaido Technology Licensing Office Co., Ltd., Japan; Chemical Biology Institute
SOURCE: PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003031628	A1	20030417	WO 2001-JP8538	20010928

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: WO 2001-JP8538 20010928

AB Human secretory sphingosine kinase 3 (SPHK3), cDNA encoding it, **recombinant expression**, and use in drug screening for and diagnosis of sphingosine-related diseases, are disclosed. A novel sphingosine kinase was purified from human and its amino acid sequence determined Its cDNA was **cloned** and **expressed** in COS-7 cells. Besides phosphorylating sphingosine to produce sphingosine-1-phosphate, it also acts on D-erythro-dihydrosphingosine, N,N-dimethyl-sphingosine, diacylglycerol, and phosphatidylinositol.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 15 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:301228 HCAPLUS

DOCUMENT NUMBER: 138:316761

TITLE: Sphingosine kinase 4 from human platelet, encoding cDNA, and use in drug screening and diagnosis

INVENTOR(S): Igarashi, Yasuyuki; Kihara, Akio

PATENT ASSIGNEE(S): Hokkaido Technology Licensing Office Co., Ltd., Japan; Chemical Biology Institute

SOURCE: PCT Int. Appl., 39 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003031627	A1	20030417	WO 2001-JP8537	20010928

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: WO 2001-JP8537 20010928

AB Human platelet-origin sphingosine kinase 4 (SPHK4), cDNA encoding it, **recombinant expression**, and use in drug screening for and diagnosis of sphingosine-related diseases, are disclosed. A novel sphingosine kinase was purified from human platelet and its amino acid sequence determined Its cDNA was **cloned** and **expressed** in E. coli.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 16 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:76920 HCAPLUS
DOCUMENT NUMBER: 138:132230
TITLE: RPK118, a novel **human sphingosine kinase**-1-binding protein
INVENTOR(S): Nakamura, Shunichi; Okada, Taro
PATENT ASSIGNEE(S): The New Industry Research Organization, Japan
SOURCE: PCT Int. Appl., 60 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003008582	A1	20030130	WO 2002-JP7352	20020719

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: JP 2001-220516 A 20010719
AB A novel sphingosine kinase 1-binding protein RPK118 isolated from human brain, its encoding cDNA, and **recombinant expression**, are disclosed. Probes and antibodies are claimed. Sphingosine kinase (SPHK) is a key enzyme catalyzing the formation of sphingosine 1 phosphate (SPP), a lipid messenger that is implicated in the regulation of a wide variety of important cellular events through intracellular as well as extracellular mechanisms. However, the mol. mechanism of the intracellular actions of SPP remains unclear. Here the authors have **cloned** a novel sphingosine kinase-1 (SPHK1)-binding protein, RPK118, by yeast two-hybrid screening. RPK118 contains several functional domains whose sequences are homologous to other known proteins including the phox homol. domain and pseudokinase 1 and 2 domains and is shown to be a member of an evolutionarily highly conserved gene family. The pseudokinase 2 domain of RPK118 is responsible for SPHK1 binding as judged by yeast two-hybrid screening and immunopptn. studies. RPK118 is also shown to co-localize with SPHK1 on early endosomes in COS7 cells **expressing** both **recombinant** proteins. Furthermore, RPK118 specifically binds to phosphatidylinositol 3-phosphate. RPK118 binds to sphingosine kinase 1 in the C-terminal side of the P-kinase domain and transports sphingosine kinase 1 to a specific site in a cell via the PX domain and the ESP domain, thereby serving as a sorting protein. These results strongly suggest that RPK118 is a novel SPHK1-binding protein that may be involved in transmitting SPP-mediated signaling into the cell.
REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 17 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:896727 HCAPLUS
DOCUMENT NUMBER: 140:74609
TITLE: Sphingosine Kinase 2 is a Nuclear Protein and Inhibits DNA Synthesis
AUTHOR(S): Igarashi, Nobuaki; Okada, Taro; Hayashi, Shun; Fujita, Toshitada; Jahangeer, Saleem; Nakamura, Shun-Ichi
CORPORATE SOURCE: Department of Molecular and Cellular Biology, Division

of Biochemistry, Kobe University Graduate School of Medicine, Kobe, 650-0017, Japan
SOURCE: Journal of Biological Chemistry (2003), 278(47), 46832-46839
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Sphingosine kinase-1 (SPHK1) is a key enzyme catalyzing the formation of an important bioactive lipid messenger, sphingosine 1-phosphate, and is implicated in the regulation of cell proliferation and antiapoptotic processes. Biol. features of another isoenzyme SPHK2, however, remain unclear. The present studies were undertaken to characterize SPHK2 by comparison with SPHK1. When SPHK2 was transiently **expressed** in various cell lines, it was localized in the nuclei as well as in the cytosol, whereas SPHK1 was distributed in the cytosol but not in the nucleus. The authors have mapped a functional nuclear localization signal (NLS) to the N-terminal region of SPHK2. The authors have observed that the **expression** of SPHK2 in various cell types causes inhibition of DNA synthesis, resulting in the cell cycle arrest at G1/S phase. The authors have also demonstrated that an NLS mutant of SPHK2, SPHK2R93E/R94E, failed to enter the nucleus and to inhibit DNA synthesis. Moreover, a fusion protein, NLS-SPHK1, where SPHK1 was fused to the NLS sequence of SPHK2 acquired the ability to enter nuclei and inhibited DNA synthesis. These results indicate that SPHK2 localizes in the nuclei and causes inhibition of DNA synthesis, and this may affect subsequent cellular events.
REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 18 OF 44 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2003460343 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14522923
TITLE: Discovery and evaluation of inhibitors of **human sphingosine kinase**.
AUTHOR: French Kevin J; Schrecengost Randy S; Lee Brian D; Zhuang Yan; Smith Staci N; Eberly Justin L; Yun Jong K; Smith Charles D
CORPORATE SOURCE: Department of Pharmacology, Penn State College of Medicine, Hershey, Pennsylvania 17033, USA.
CONTRACT NUMBER: R24 CA788243 (NCI)
SOURCE: Cancer research, (2003 Sep 15) 63 (18) 5962-9.
Journal code: 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200312
ENTRY DATE: Entered STN: 20031003
Last Updated on STN: 20031218
Entered Medline: 20031204
AB Sphingolipid-metabolizing enzymes control the dynamic balance of the cellular levels of bioactive lipids, including the proapoptotic compound ceramide and the proliferative compound sphingosine 1-phosphate. Accumulating evidence indicates that sphingosine kinase (SK) plays a pivotal role in regulating tumor growth and that SK can act as an oncogene. Despite the importance of SK for cell proliferation, pharmacological inhibition of SK is an untested means of treating cancer because of the current lack of nonlipid inhibitors of this enzyme. To further assess the involvement of SK in human tumors, levels of RNA for SK in paired samples of cDNA prepared from tumors and normal adjacent tissue were analyzed. **Expression** of SK RNA was significantly elevated in a variety of solid tumors, compared with normal tissue from the same patient. To identify and evaluate inhibitors of SK, a medium throughput

assay for **recombinant** human SK fused to glutathione S-transferase was developed, validated, and used to screen a library of synthetic compounds. A number of novel inhibitors of human SK were identified, and several representative compounds were characterized in detail. These compounds demonstrated activity at sub- to micromolar concentrations, making them more potent than any other reported SK inhibitor, and were selective toward SK compared with a panel of human lipid and protein kinases. Kinetic studies revealed that the compounds were not competitive inhibitors of the ATP-binding site of SK. The SK inhibitors were antiproliferative toward a panel of tumor cell lines, including lines with the multidrug resistance phenotype because of overexpression of either P-glycoprotein or multidrug resistance phenotype 1, and were shown to inhibit endogenous human SK activity in intact cells. Furthermore, each inhibitor induced apoptosis concomitant with tumor cell cytotoxicity. Methods for the synthesis of a series of aurone inhibitors of SK were established, and a prototypical dihydroxyaurone was found to have moderate antitumor activity in vivo in the absence of overt toxicity to the mice. These compounds are the first examples of nonlipid inhibitors of SK with in vivo antitumor activity and so provide leads for additional development of inhibitors of this important molecular target.

L6 ANSWER 19 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:318819 HCAPLUS
DOCUMENT NUMBER: 139:34620
TITLE: Sphingosine kinase-dependent migration of immature dendritic cells in response to neurotoxic prion protein fragment
AUTHOR(S): Kaneider, Nicole C.; Kaser, Arthur; Dunzendorfer, Stefan; Tilg, Herbert; Wiedermann, Christian J.
CORPORATE SOURCE: Division of General Internal Medicine, Department of Internal Medicine, University of Innsbruck, Innsbruck, A-6020, Austria
SOURCE: Journal of Virology (2003), 77(9), 5535-5539
CODEN: JOVIAM; ISSN: 0022-538X
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The concept that circulating dendritic cells mediate neuroinvasion in transmissible spongiform encephalopathies received strong support from recent observations that prion protein is **expressed** in myeloid dendritic cells. The authors observed that prion protein fragment 106-126 is a chemoattractant for monocyte-derived immature but not mature dendritic cells. Signaling events in chemotaxis involved enzymes downstream of Gq protein and were inhibited by blockade of sphingosine kinase, suggesting transactivation of sphingosine-1-phosphate-dependent cell motility by prion protein.
REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 20 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:810828 HCAPLUS
DOCUMENT NUMBER: 140:71238
TITLE: Sphingosine kinase transmits estrogen signaling in human breast cancer cells
AUTHOR(S): Sukocheva, Olga A.; Wang, Lijun; Albanese, Nathaniel; Pitson, Stuart M.; Vadas, Mathew A.; Xia, Pu
CORPORATE SOURCE: Signal Transduction Laboratory, Division of Human Immunology, Hanson Institute, Institute of Medical and Veterinary Science and University of Adelaide, Adelaide, 5000, Australia
SOURCE: Molecular Endocrinology (2003), 17(10), 2002-2012
CODEN: MOENEN; ISSN: 0888-8809
PUBLISHER: Endocrine Society
DOCUMENT TYPE: Journal

LANGUAGE: English

AB Current understanding of cytoplasmic signaling pathways that mediate estrogen action in human breast cancer is incomplete. Here we report that treatment with 17β-estradiol (E2) activates a novel signaling pathway via activation of sphingosine kinase (SphK) in MCF-7 breast cancer cells. We found that E2 has dual actions to stimulate SphK activity, i.e. a rapid and transient activation mediated by putative membrane G protein-coupled estrogen receptors (ER) and a delayed but prolonged activation relying on the transcriptional activity of ER. The E2-induced SphK activity consequently activates downstream signal cascades including intracellular Ca2+ mobilization and Erk1/2 activation. Enforced **expression** of human SphK type 1 gene in MCF-7 cells resulted in increases in SphK activity and cell growth. Moreover, the E2-dependent mitogenesis were highly promoted by SphK overexpression as determined by colony growth in soft agar and solid focus formation. In contrast, **expression** of SphKG82D, a dominant-neg. mutant SphK, profoundly inhibited the E2-mediated Ca2+ mobilization, Erk1/2 activity and neoplastic cell growth. Thus, our data suggest that SphK activation is an important cytoplasmic signaling to transduce estrogen-dependent mitogenic and carcinogenic action in human breast cancer cells.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 21 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2003:189342 HCAPLUS

DOCUMENT NUMBER: 139:52761

TITLE: Synthesis of fluorescence-labeled sphingosine and sphingosine 1-phosphate; effective tools for sphingosine and sphingosine 1-phosphate behavior

AUTHOR(S): Hakogi, Toshikazu; Shigenari, Toshihiko; Katsumura, Shigeo; Sano, Takamitsu; Kohno, Takayuki; Igarashi, Yasuyuki

CORPORATE SOURCE: School of Science and Technology, Kwansei Gakuin University, Sanda, Hyogo, 669-1337, Japan

SOURCE: Bioorganic & Medicinal Chemistry Letters (2003), 13(4), 661-664
CODEN: BMCLE8; ISSN: 0960-894X

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

OTHER SOURCE(S): CASREACT 139:52761

AB A fluorescence-labeled sphingosine (I; R = H) and sphingosine 1-phosphate (I; R = PO3H2) have been successfully synthesized from the oxazolidinone Me ester derived from glycidol via monoalkylation and the stereoselective reduction of the resulting ketone. The labeled sphingosine was converted into its phosphate by treatment with sphingosine kinase 1 (SPHK1) from mouse, and in platelets, and it was incorporated into the Chinese Hamster Ovarian (CHO) cells. In addition, MAPK was activated by NBD-Sph-1-P through Edg-1, Sph-1-P receptor.

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 22 OF 44 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:1003240 SCISEARCH

THE GENUINE ARTICLE: 741TT

TITLE: Sphingosine-1-phosphate formation activates phosphatidylinositol-4 kinase in basolateral membranes from kidney cells: Crosstalk in cell signaling through sphingolipids and phospholipids

AUTHOR: Einicker-Lamas M; Wenceslau L D; Bernardo R R; Nogaroli L; Guilherme A; Oliveira M M; Vieyra A (Reprint)

CORPORATE SOURCE: Univ Fed Rio de Janeiro, Inst Biofis Carlos Chagas Filho, BR-21941590 Rio De Janeiro, Brazil (Reprint); Univ

Massachusetts, Sch Med, Worcester, MA 01605 USA
COUNTRY OF AUTHOR: Brazil; USA
SOURCE: JOURNAL OF BIOCHEMISTRY, (OCT 2003) Vol. 134, No. 4, pp. 529-536.
ISSN: 0021-924X.
PUBLISHER: JAPANESE BIOCHEMICAL SOC, ISHIKAWA BLDG-3F, 25-16 HONGO-5-CHOME, BUNKYO-KU, TOKYO, 113, JAPAN.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 66
ENTRY DATE: Entered STN: 8 Dec 2003
Last Updated on STN: 8 Dec 2003
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Sphingosine-1-phosphate (SIP) and phosphatidylinositol-4 phosphate [PtdIns(4)P] are important second messengers in various cellular processes. Here, we show that SIP and PtdIns(4)P are formed in purified basolateral membranes (BLM) derived from kidney proximal tubules, indicating the presence of a plasma membrane associated SPK (BLM-SPK) and phosphatidylinositol-4 kinase (PI-4K). We observed that SIP synthesis is linear with time, dependent on protein concentration, and saturable in the presence of increasing concentrations of sphingosine. Different from the observations on cytosolic SPKs, the formation of SIP by BLM-SPK is Mg2+-independent and insensitive to the classical inhibitor of the cytosolic SPKs, DL-threo-dihydrosphingosine. With sphingosine as substrate, the enzyme shows cooperative kinetics (n = 3.4) with a K-0.5 value of 0.12 mM, suggesting that BLM-SPK is different from the previously characterized cytosolic SPK. The formation of PtdIns(4)P markedly inhibits BLM-SPK activity. Conversely, a strong activation of PtdIns(4)P synthesis by the formation of SIP is observed. Taken together, these results indicate that (i) basolateral membranes from kidney cells harbor a SPK activity that potentially regulates renal epithelium function, and (ii) the formation of SIP mediated by SPK enhances PI-4K activity, while PtdIns(4)P in turn inhibits SPK, suggesting an interplay between these lipid signaling molecules. These findings suggest the possibility of crosstalk between sphingolipids and glycerolipids, which might be involved in the regulation of transepithelial fluxes across the BLM of kidney cells.

L6 ANSWER 23 OF 44 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2003498617 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14575709
TITLE: Identification of functional nuclear export sequences in **human sphingosine kinase** 1.
AUTHOR: Inagaki Yuichi; Li Pei-Yun; Wada Atsushi; Mitsutake Susumu; Igarashi Yasuyuki
CORPORATE SOURCE: Department of Biomembrane and Biofunctional Chemistry, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.
SOURCE: Biochemical and biophysical research communications, (2003 Nov 7) 311 (1) 168-73.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200401
ENTRY DATE: Entered STN: 20031025
Last Updated on STN: 20040122
Entered Medline: 20040121

AB Sphingosine kinase (SPHK) is an enzyme that phosphorylates sphingosine to form sphingosine 1-phosphate (S1P). Human SPHK1 (hSPHK1) was localized predominantly in the cytoplasm when transiently **expressed** in Cos7 cells. In this study, we have found two functional nuclear export signal (NES) sequences in the middle region of hSPHK1. Deletion and

mutagenesis studies revealed that the cytoplasmic localization of SPHK1 depends on its nuclear export, directed by the NES. Furthermore, upon treatment with leptomycin B, a specific inhibitor of the nuclear export receptor CRM1, a marked nuclear accumulation of hSPHK1 was observed, indicating that hSPHK1 shuttles between the cytoplasm and the nucleus. Our results provide the first evidence of the active nuclear export of SPHK1 and suggest it is mediated by a CRM1-dependent pathway.

L6 ANSWER 24 OF 44 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:138843 BIOSIS
DOCUMENT NUMBER: PREV200400140829
TITLE: Regulation of sphingosine kinase 1 gene **expression** by protein kinase C in a human leukemia cell line, MEG-01.
AUTHOR(S): Nakade, Yusuke; Banno, Yoshiko; T-Koizumi, Keiko; Hagiwara, Kazumi; Sobue, Sayaka; Koda, Masahiro; Suzuki, Motoshi; Kojima, Tetsuhito; Takagi, Akira; Asano, Haruhiko; Nozawa, Yoshinori; Murate, Takashi [Reprint Author]
CORPORATE SOURCE: Graduate School of Medicine, School of Health Sciences, Nagoya University, Daiko-Minami 1-1-20, Higashi, 461-8673, Nagoya, Japan
murate@met.nagoya-u.ac.jp
SOURCE: Biochimica et Biophysica Acta, (30 December 2003) Vol. 1635, No. 2-3, pp. 104-116. print.
ISSN: 0006-3002 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Mar 2004
Last Updated on STN: 10 Mar 2004

AB The prolonged treatment with phorbol 12-myristate 13-acetate (PMA) of a human megakaryoblastic leukemia cell line, MEG-01, induced increase of sphingosine kinase (SPHK) enzyme activity and SPHK1 protein **expression** as well as SPHK1 message. Protein kinase C (PKC) inhibitor prevented the PMA-induced SPHK1 gene **expression**. To elucidate the regulatory mechanism of this gene **expression**, we examined the promoter area (distal to the first exon) and its binding proteins. Luciferase analyses showed that the area of 300 bp from the first exon was sufficient for PMA-responsiveness, and that specificity protein 1 (Sp1)- and two activator protein 2 (AP-2)-binding motifs within this area were necessary for responsiveness. Inhibitors for PKCTM and MEK1 decreased this PMA-induced promoter activity. Electrophoresis mobility shift assay (EMSA) showed that Sp1 protein was originally bound to the Sp1 site and that two additional bands bound to the two AP-2 motifs were observed only when stimulated with PMA in MEG-01 cells. The appearance of these bands resulted from binding to an unknown protein rather than AP-2. These results indicated that PMA up-regulates SPHK1 gene **expression** through PMA-responsive elements of the 5' promoter area of the gene, and suggested that PMA-mediated SPHK1 gene **expression** would be mediated via PKC- and ERK-dependent signal transduction pathway by binding the transcription factor to AP-2 motifs.

L6 ANSWER 25 OF 44 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2003-10480 BIOTECHDS
TITLE: Modulating cytokine- or tumor necrosis factor-induced cellular activity, useful for treating or preventing a neoplastic condition, comprises modulating an intracellular sphingosine kinase-dependent signaling mechanism; protein-induced cellular activity modulation and agonist and antagonist for use in disease therapy
AUTHOR: XIA P; WANG L; VADAS M; GAMBLE J; MORETTI P; PITSON S
PATENT ASSIGNEE: MEDVET SCI PTY LTD
PATENT INFO: WO 2002098458 12 Dec 2002
APPLICATION INFO: WO 2002-AU710 3 Jun 2002
PRIORITY INFO: AU 2001-9759 27 Dec 2001; AU 2001-5521 7 Jun 2001

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-201282 [19]

AB DERWENT ABSTRACT:

NOVELTY - Modulating cytokine-induced or tumor necrosis factor (TNF)-induced cellular activity, comprises contacting the cell with an agent under conditions sufficient to modulate the interaction of sphingosine kinase with a TNF receptor-associated factor (TRAF), preferably TRAF2, where inducing the association up-regulates cellular activity, and inhibiting the association down-regulates cellular activity.

DETAILED DESCRIPTION - Modulating cytokine-induced or tumor necrosis factor (TNF)-induced cellular activity, comprises contacting the cell with an agent for a time and under conditions sufficient to modulate the interaction of sphingosine kinase with a TNF receptor-associated factor (TRAF), preferably TRAF2, where inducing or agonizing the association up-regulates the cellular activity, and inhibiting or antagonizing the association down-regulates the cellular activity. INDEPENDENT CLAIMS are included for the following: (1) treating and/or preventing a condition of aberrant, unwanted or inappropriate cytokine-induced or tumor necrosis factor (TNF)-induced cellular activity in a mammal; (2) detecting an agent capable of modulating the interaction of TRAF with sphingosine kinase or its functional equivalent or derivative; (3) analyzing, designing and/or modifying an agent capable of interacting with the TRAF binding site of sphingosine kinase or its derivative and modulating at least one functional activity associated with the sphingosine kinase; (4) an agent described or identified in the methods cited above; and (5) a pharmaceutical condition comprising the modulatory agent described in the methods above, and one or more pharmaceutical carriers and/or diluents;

BIOTECHNOLOGY - Preferred Methods: The tumor necrosis factor (TNF)-induced cellular activity is the induction of anti-apoptotic characteristics, and modulation is down-regulation of the interaction of sphingosine kinase with TNF receptor-associated factor (TRAF). The TNF-induced cellular activity is the induction of pro-inflammatory, and the induction is down-regulation of the interaction of sphingosine kinase with TRAF. The agent binds, links or associates with the C-terminal region of sphingosine kinase, where the C-terminal region is the amino acid sequence of Pro-Pro-Glu Glu (I). The sphingosine kinase is preferably **human sphingosine kinase**, and the C-terminal region is the sequence of (I) at amino acid residue numbers 379-382 of a fully defined sequence of 384 amino acids (S1) given in the specification. Treating and/or preventing a condition of aberrant, unwanted or inappropriate cytokine-induced cellular activity in a mammal, comprises administering to the mammal an agent that modulates the interaction of sphingosine kinase with a TRAF, where inducing or agonizing the association up-regulates the cellular activity, and inhibiting or antagonizing the association down-regulates the cellular activity. Treating and/or preventing a condition of aberrant, unwanted or inappropriate TNF-induced cellular activity in a mammal, comprises administering to the mammal an agent that modulates the interaction of sphingosine kinase with a TRAF, preferably TRAF2, where inducing or agonizing the association up-regulates the cellular activity, and inhibiting or antagonizing the association down-regulates the cellular activity. The mammal is preferably human and the condition is a neoplastic condition. Detecting an agent capable of modulating the interaction of TRAF with sphingosine kinase or its functional equivalent or derivative, comprises contacting a cell or its extract containing the sphingosine kinase and TRAF or its functional equivalent or derivative with a putative agent, and detecting an altered **expression** phenotype associated with the interaction. TRAF is preferably TRAF2. The altered **expression** phenotype is an altered apoptosis profile or is modulation of the functional activity of sphingosine kinase. Analyzing, designing and/or modifying an agent capable of interacting with the TRAF binding site of sphingosine kinase or its derivative and

modulating at least one functional activity associated with the sphingosine kinase, comprises contacting the sphingosine kinase or its derivative with a putative agent and assessing the degree of interactive complementarity of the agent with the binding site. The TRAF binding site is the C-terminal region of sphingosine kinase, which is a **human sphingosine kinase**, and the C-terminal region is the sequence of (I) at amino acid residue numbers 379-382 of the sequence of S1.

ACTIVITY - Cytostatic; Antiinflammatory; Antirheumatic; Antiarthritic. No biological data given.

MECHANISM OF ACTION - Sphingosine Kinase Inhibitor; Sphingosine Kinase Stimulator; TRAF Agonist 2; TRAF Antagonist 2.

USE - The agent is useful for manufacturing a medicament for treating a mammal with a condition of aberrant, unwanted or inappropriate cytokine-induced or tumor necrosis factor (TNF)-induced cellular activity (claimed). The methods are useful for modulating cytokine-induced or TNF-induced cellular activity, or for treating or preventing a condition of aberrant, unwanted or inappropriate cytokine-induced or TNF-induced cellular activity in a mammal, such as neoplastic condition or inflammation (e.g. rheumatoid arthritis).

ADMINISTRATION - Dosage is about 0.1-1 mg/kg/day. Administration may be oral, intravenous, intraperitoneal, intramuscular, subcutaneous, intradermal, rectal, intratracheal, intracranial, intraocular, intrathecal, intracerebral, or intranasal.

EXAMPLE - Human embryonic kidney cell line 293T was transiently transfected with wild type TNF receptor-associated factor-2 (TRAF2), a dominant-negative TRAF2, or an empty vector. Over-**expression** of TRAF2 not only enhanced TNF-induced sphingosine kinase but also itself was capable of activating sphingosine kinase by two-fold compared with control transfectants. Immunoblotting assay showed equivalent **expression** levels of the transgenes in the presence or absence of TNF stimulation. (96 pages)

L6 ANSWER 26 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:276032 HCAPLUS
DOCUMENT NUMBER: 136:304111
TITLE: Regulation of **human sphingosine kinase**-like protein and uses in diagnosis, therapy and drug screening
INVENTOR(S): Kossida, Sophia; Encinas, Jeffrey
PATENT ASSIGNEE(S): Bayer Aktiengesellschaft, Germany
SOURCE: PCT Int. Appl., 120 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002028906	A2	20020411	WO 2001-EP11516	20011005
WO 2002028906	A3	20021114		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002023593	A5	20020415	AU 2002-23593	20011005
EP 1326986	A2	20030716	EP 2001-986303	20011005
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				

IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

JP 2004510429	T2	20040408	JP 2002-532488		20011005
PRIORITY APPLN. INFO.:			US 2000-238005P	P	20001006
			US 2001-314113P	P	20010823
			WO 2001-EP11516	W	20011005

AB Reagents which regulate **human sphingosine kinase**-like protein activity and reagents which bind to **human sphingosine kinase**-like protein gene products can be used to regulate intracellular signaling intracellular signaling and consequently cell proliferation and apoptosis. Such regulation is particularly useful for treating cancer, allergies including but not limited to asthma, autoimmune diseases such as rheumatoid arthritis, and central and peripheral nervous system disorders, such as Parkinson's disease.

L6 ANSWER 27 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:256587 HCAPLUS
DOCUMENT NUMBER: 136:291008
TITLE: Methods and compositions for screening modulators of lipid kinases
INVENTOR(S): Normant, Emmanuel; Melendez, Alirio; Casamitjana, Olivier; Moreau, Francois
PATENT ASSIGNEE(S): Warner-Lambert Company, USA
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002027318	A1	20020404	WO 2001-EP11250	20010928
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1195604	A1	20020410	EP 2000-402684	20000929
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
CA 2423889	AA	20020404	CA 2001-2423889	20010928
AU 2001089939	A5	20020408	AU 2001-89939	20010928
EP 1195605	A1	20020410	EP 2001-402500	20010928
EP 1195605	B1	20040331		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 2002042091	A1	20020411	US 2001-964860	20010928
US 6723525	B2	20040420		
JP 2004509638	T2	20040402	JP 2002-530646	20010928
AT 263373	E	20040415	AT 2001-402500	20010928
PT 1195605	T	20040831	PT 2001-402500	20010928
ES 2218351	T3	20041116	ES 2001-1402500	20010928
PRIORITY APPLN. INFO.:			EP 2000-402684 A	20000929
			EP 2000-2000402684 A	20000929
			WO 2001-EP11250 W	20010928

AB The present invention relates to methods of screening compds. that modulate lipid kinases activity. The invention is more preferably based on the SPA technol. to screen compds. that modulate the activity of lipid kinases, in particular membrane lipid kinases, more specifically

sphingosine kinases. The invention also includes compns., products, kits, etc. for use in performing the above methods, as well as the compds. identified by said methods, and their uses.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 28 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:10690 HCAPLUS
DOCUMENT NUMBER: 136:81963
TITLE: Molecular variants of mammalian sphingosine kinase with reduced catalytic activity and therapeutic uses thereof
INVENTOR(S): Pitson, Stuart; Moretti, Paul; Zebol, Julia; Xia, Pu; Gamble, Jennifer; Vadas, Mathew; D'Andrea, Richard; Wattenberg, Binks
PATENT ASSIGNEE(S): Medvet Science Pty. Ltd., Australia
SOURCE: PCT Int. Appl., 104 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002000887	A1	20020103	WO 2001-AU730	20010620
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2414210	AA	20020103	CA 2001-2414210	20010620
AU 2001065699	A5	20020108	AU 2001-65699	20010620
EP 1299548	A1	20030409	EP 2001-942904	20010620
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2004500903	T2	20040115	JP 2002-506202	20010620
BR 2001012059	A	20040727	BR 2001-12059	20010620
NZ 523343	A	20050324	NZ 2001-523343	20010620
NO 2002006265	A	20030224	NO 2002-6265	20021227
ZA 2003000214	A	20040408	ZA 2003-214	20030108

PRIORITY APPLN. INFO.:

AU 2000-8408	A	20000628
AU 2000-8699	A	20000711
AU 2000-9980	A	20000908
AU 2001-2749	A	20010129
WO 2001-AU730	W	20010620

AB The present invention relates generally to a sphingosine kinase variant and to derivs., analogs, chemical equivalent and mimetics thereof exhibiting reduced catalytic activity and, more particularly, to sphingosine kinase variants which exhibit a reduced capacity to phosphorylate sphingosine to sphingosine-1-phosphate. The present invention also contemplates genetic sequences encoding said sphingosine kinase variants and derivs., analogs and mimetics thereof. The variants of the present invention are useful in a range of therapeutic and prophylactic applications. Site-directed mutagenesis of a putative ATP-binding site (glycine in position 82 to aspartic acid, G82D) resulted in a catalytically inactive sphingosine kinase (SK) for phosphorylating sphingosine to sphingosine-1-phosphate. The G82D SK is **expressed**, as shown by Western blots, and does not suppress endogenous cellular SK activity. However, G82D SK decreases activation of sphingosine kinase activity after treatment of cells with

agents such as TNF, IL-1, and PMA and it inhibits SK activity that is stimulated by the Ras oncogene. Another mutant G82A (glycine at position 82 substituted with alanine) retains about 5% of the wild-type level of catalytic activity. Anal. of substrate kinetics of G82A SK shows low affinity for ATP but wild-type affinity for sphingosine.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 29 OF 44 MEDLINE on STN DUPLICATE 5

ACCESSION NUMBER: 2002731982 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12393916
TITLE: The nucleotide-binding site of **human sphingosine kinase** 1.
AUTHOR: Pitson Stuart M; Moretti Paul A B; Zebol Julia R; Zareie Reza; Derian Claudia K; Darrow Andrew L; Qi Jenson; D'Andrea Richard J; Bagley Christopher J; Vadas Mathew A; Wattenberg Binks W
CORPORATE SOURCE: Hanson Institute, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide SA 5000, Australia.. stuart.pitson@imvs.sa.gov.asu
SOURCE: Journal of biological chemistry, (2002 Dec 20) 277 (51) 49545-53. Electronic Publication: 2002-10-18. Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200302
ENTRY DATE: Entered STN: 20021227
Last Updated on STN: 20030214
Entered Medline: 20030212

AB Sphingosine kinase catalyzes the formation of sphingosine 1-phosphate, a lipid second messenger that has been implicated in a number of agonist-driven cellular responses including mitogenesis, anti-apoptosis, and **expression** of inflammatory molecules. Despite the importance of sphingosine kinase, very little is known regarding its structure or mechanism of catalysis. Moreover, sphingosine kinase does not contain recognizable catalytic or substrate-binding sites, based on sequence motifs found in other kinases. Here we have elucidated the nucleotide-binding site of **human sphingosine kinase** 1 (hSK1) through a combination of site-directed mutagenesis and affinity labeling with the ATP analogue, FSBA. We have shown that Gly(82) of hSK1 is involved in ATP binding since mutation of this residue to alanine resulted in an enzyme with an approximately 45-fold higher K(m)((ATP)). We have also shown that Lys(103) is important in catalysis since an alanine substitution of this residue ablates catalytic activity. Furthermore, we have shown that this residue is covalently modified by FSBA. Our data, combined with amino acid sequence comparison, suggest a motif of SGDGX(17-21)K is involved in nucleotide binding in the sphingosine kinases. This motif differs in primary sequence from all previously identified nucleotide-binding sites. It does, however, share some sequence and likely structural similarity with the highly conserved glycine-rich loop, which is known to be involved in anchoring and positioning the nucleotide in the catalytic site of many protein kinases.

L6 ANSWER 30 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:720587 HCAPLUS
DOCUMENT NUMBER: 138:132764
TITLE: **Cloning** and Characterization of a Protein Kinase A Anchoring Protein (AKAP)-related Protein That Interacts with and Regulates Sphingosine Kinase 1 Activity
AUTHOR(S): Lacana, Emanuela; Maceyka, Michael; Milstien, Sheldon; Spiegel, Sarah

CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Georgetown University Medical School, Washington, DC, 20007, USA
SOURCE: Journal of Biological Chemistry (2002), 277(36), 32947-32953
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that has novel dual actions. S1P is the ligand for a family of G protein-coupled receptors known as S1PRs that mediate various physiol. functions. Growth factors rapidly activate sphingosine kinase type 1 (SPHK1) resulting in phosphorylation of sphingosine to form S1P, which plays important roles in cell growth regulation and protection from apoptosis. However, little is known of the mechanism(s) by which SPHK activity is regulated. Using a yeast two-hybrid screening approach, we **cloned** a 3-kb cDNA encoding a SPHK1-interacting protein (SKIP). BLAST anal. revealed that SKIP corresponded to the C-terminal region of a larger (.apprx.7 kb) cDNA that encoded a protein with a high degree of similarity to a family of protein kinase A anchor proteins (AKAP). In confirmation of the yeast two-hybrid assay, glutathione S-transferase (GST)-SPHK1 specifically pulled down SKIP, whereas GST did not. Moreover, immunopptn. of in vitro translated SPHK1 and SKIP revealed that SKIP and SPHK1 are tightly associated Furthermore, SKIP overexpression in NIH 3T3 fibroblasts reduced SPHK1 activity and interfered with its biol. functions. The apoptotic-sparing effect of SPHK1 against serum deprivation was reduced when co-transfected with SKIP. In addition, SPHK1-enhanced cell proliferation was also abolished by SKIP, with a corresponding decrease in activation of ERK. Taken together, these results indicate that SKIP is a novel protein likely to play a regulatory role in the modulation of SPHK1 activity.

REFERENCE COUNT: 59 THERE ARE 59 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 31 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2002:834308 HCAPLUS
DOCUMENT NUMBER: 138:117912
TITLE: Sphingosine kinase mediates vascular endothelial growth factor-induced activation of Ras and mitogen-activated protein kinases
AUTHOR(S): Shu, Xiaodong; Wu, Weicheng; Mosteller, Raymond D.; Broek, Daniel
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Norris Comprehensive Cancer Center, Keck School of Medicine at the University of Southern California, Los Angeles, CA, 90089, USA
SOURCE: Molecular and Cellular Biology (2002), 22(22), 7758-7768
CODEN: MCEBD4; ISSN: 0270-7306
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Vascular endothelial growth factor (VEGF) signaling is critical to the processes of angiogenesis and tumor growth. Here, evidence is presented for VEGF stimulation of sphingosine kinase (SPK) that affects not only endothelial cell signaling but also tumor cells **expressing** VEGF receptors. VEGF or phorbol 12-myristate 13-acetate treatment of the T24 bladder tumor cell line resulted in a time- and dose-dependent stimulation of SPK activity. In T24 cells, VEGF treatment reduced cellular sphingosine levels while raising that of sphingosine-1-phosphate. VEGF stimulation of T24 cells caused a slow and sustained accumulation of Ras-GTP and phosphorylated extracellular signal-regulated kinase

(phospho-ERK) compared with that after EGF treatment. Small interfering RNA (siRNA) that targets SPK1, but not SPK2, blocks VEGF-induced accumulation of Ras-GTP and phospho-ERK in T24 cells. In contrast to EGF stimulation, VEGF stimulation of ERK1/2 phosphorylation was unaffected by dominant-neg. Ras-N17. Raf kinase inhibition blocked both VEGF- and EGF-stimulated accumulation of phospho-ERK1/2. Inhibition of SPK by pharmacol. inhibitors, a dominant-neg. SPK mutant, or siRNA that targets SPK blocked VEGF, but not EGF, induction of phospho-ERK1/2. We conclude that VEGF induces DNA synthesis in a pathway which sequentially involves protein kinase C (PKC), SPK, Ras, Raf, and ERK1/2. These data highlight a novel mechanism by which SPK mediates signaling from PKC to Ras in a manner independent of Ras-guanine nucleotide exchange factor.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 32 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:857385 HCAPLUS

DOCUMENT NUMBER: 138:120688

TITLE: Sphingosine Kinase Type 1 Promotes Estrogen-Dependent Tumorigenesis of Breast Cancer MCF-7 Cells

AUTHOR(S): Nava, Victor E.; Hobson, John Peyton; Murthy, Shvetha; Milstien, Sheldon; Spiegel, Sarah

CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, DC, 20007, USA

SOURCE: Experimental Cell Research (2002), 281(1), 115-127
CODEN: ECREAL; ISSN: 0014-4827

PUBLISHER: Elsevier Science

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The sphingolipid metabolite, sphingosine-1-phosphate (S1P), formed by phosphorylation of sphingosine, has been implicated in cell growth, suppression of apoptosis, and angiogenesis. In this study, we have examined the contribution of intracellular S1P to tumorigenesis of breast adenocarcinoma MCF-7 cells. Enforced **expression** of sphingosine kinase type 1 (SPHK1) increased S1P levels and blocked MCF-7 cell death induced by anti-cancer drugs, sphingosine, and TNF-α. SPHK1 also conferred a growth advantage, as determined by proliferation and growth in soft agar, which was estrogen dependent. While both ERK and Akt have been implicated in MCF-7 cell growth, SPHK1 stimulated ERK1/2 but had no effect on Akt. Surprisingly, parental growth of MCF-7 cells was only weakly stimulated by S1P or dihydro-S1P, ligands for the S1P receptors which usually mediate growth effects. When injected into mammary fat pads of ovariectomized nude mice implanted with estrogen pellets, MCF-7/SPHK1 cells formed more and larger tumors than vector transfectants with higher microvessel d. in their periphery. Collectively, our results suggest that SPHK1 may play an important role in breast cancer progression by regulating tumor cell growth and survival.

REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 33 OF 44 MEDLINE on STN DUPLICATE 6

ACCESSION NUMBER: 2002228759 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11923095

TITLE: 1-O-Hexadecyl-2-desoxy-2-amino-sn-glycerol, a substrate for **human sphingosine kinase.**

AUTHOR: Gijsbers Sofie; Asselberghs Stanny; Herdewijn Piet; Van Veldhoven Paul P

CORPORATE SOURCE: Katholieke Universiteit Leuven, Faculteit Geneeskunde, Departement Moleculaire Celbiologie, Afdeling Farmakologie, Herestraat, Belgium.

SOURCE: Biochimica et biophysica acta, (2002 Jan 30) 1580 (1) 1-8.
Journal code: 0217513. ISSN: 0006-3002.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020423
Last Updated on STN: 20020528
Entered Medline: 20020524

AB The substrate specificity of **human sphingosine kinase** was investigated using a bacterially **expressed** poly(His)-tagged protein. Only the D-erythro isomer of the sphingoid bases, sphinganine and sphingenine, was effectively phosphorylated. Long chain 1-alkanols, alkane-1,2-diols, 2-amino-1-alkanol or 1-amino-2-alkanol and short chain 2-amino-1,3-alkanediols were very poor substrates, indicating that the kinase is recognizing the chain length and the position of the amino and secondary hydroxy group. A free hydroxy group at carbon 3 is not a prerequisite, however, since 1-O-hexadecyl-2-desoxy-2-amino-sn-glycerol was an efficient substrate with an apparent K(m) value of 3.8 microM (versus 15.7 microM for sphingenine). This finding opens new perspectives to design sphingosine kinase inhibitors. It also calls for some caution since it cannot be excluded that this ether lipid analogue is formed from precursors that are frequently used in research on platelet activating factor or from phospholipid analogues which are less prone to degradation.

L6 ANSWER 34 OF 44 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 7
ACCESSION NUMBER: 2001-09987 BIOTECHDS
TITLE: New **human sphingosine-kinase** type-I gene for screening drug candidates particularly inhibitors used for preventing or treating e.g. atherosclerosis, thrombosis, asthma and diabetes; baculo virus vector, plasmid pcDNA3, plasmid pFastBacHTa, plasmid pFLAG or plasmid pCMV-mediated gene transfer, **expression** in host cell, antibody and DNA primer for drug screening
AUTHOR: Allen J; Gosink M; Melendez A J; Takacs L
PATENT ASSIGNEE: Warner
LOCATION: Morris Plains, NJ, USA.
PATENT INFO: WO 2001031029 3 May 2001
APPLICATION INFO: WO 2000-EP9498 27 Oct 2000
PRIORITY INFO: US 2000-180525 7 Feb 2000; US 1999-162307 28 Oct 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-300510 [31]

AB A purified or isolated DNA encoding a **human sphingosine -kinase** (hSK), which together with its encoded protein are applicable in drug screening particularly inhibitors for preventing or treating e.g. atherosclerosis, thrombosis, asthma and diabetes, is claimed. Also claimed are: a purified or isolated DNA encoding hSK protein having a specified sequence; a DNA having a specified 240 bp sequence; a **recombinant** vector containing the DNA; a **recombinant** host cell containing the DNA or the **recombinant** vector; an antisense oligonucleotide of the specified sequences; a transgenic animal (mouse) containing the DNA; a purified protein with the sequence of hSK; amplifying a DNA encoding hSK using a hSK-specific DNA primer; a kit for amplification containing the DNA primers and reagents for performing the amplification; producing a **recombinant** protein with a specified 384 amino acid sequence by culturing the **recombinant** host cell and recovering the protein from the culture; an antibody specific for the protein; and screening for drug candidates, particularly inhibitors of hSK. The protein is useful in drug screening assays. (90pp)

L6 ANSWER 35 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:747808 HCAPLUS
DOCUMENT NUMBER: 135:300491
TITLE: **Cloning, expression** and therapeutic use of mammalian sphingosine kinase type 2 isoforms
INVENTOR(S): Spiegel, Sarah; Kohama, Takafumi
PATENT ASSIGNEE(S): Sankyo Company, Ltd., Japan; Georgetown University
SOURCE: PCT Int. Appl., 117 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001074837	A1	20011011	WO 2001-US9664	20010326
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2404965	AA	20011011	CA 2001-2404965	20010326
US 2002042101	A1	20020411	US 2001-817676	20010326
US 6800470	B2	20041005		
EP 1268509	A1	20030102	EP 2001-924340	20010326
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2004500117	T2	20040108	JP 2001-572526	20010326
BR 2001009827	A	20040706	BR 2001-9827	20010326
NO 2002004727	A	20021203	NO 2002-4727	20021002
ZA 2002007930	A	20040127	ZA 2002-7930	20021002
US 2004203104	A1	20041014	US 2004-830677	20040422

PRIORITY APPLN. INFO.:
US 2000-194318P P 20000403
US 2001-817676 A 20010326
WO 2001-US9664 W 20010326

AB The present invention concerns nucleic acids encoding mouse and **human sphingosine kinase** type 2 isoforms, methods for detecting agents or drugs which inhibit or promote sphingosine activity and therapeutic agents containing peptides or antibodies to peptides encoded by such nucleic acids. Amino acid and encoding cDNA sequences of the mouse and **human sphingosine kinase** type 2 isoforms are provided.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 36 OF 44 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2001:618152 HCAPLUS
DOCUMENT NUMBER: 135:192176
TITLE: **Cloning**, sequence and therapeutic and diagnostic use of sphingosine kinases from human, rat and mouse
INVENTOR(S): Rastelli, Luca
PATENT ASSIGNEE(S): Curagen Corporation, USA; Genentech, Inc.
SOURCE: PCT Int. Appl., 107 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001060990	A2	20010823	WO 2001-US4789	20010214
WO 2001060990	A3	20020321		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2402148	AA	20010823	CA 2001-2402148	20010214
US 2002082203	A1	20020627	US 2001-784810	20010214
US 6858427	B2	20050222		
EP 1257637	A2	20021120	EP 2001-910701	20010214
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
US 2005123942	A1	20050609	US 2004-876281	20040624

PRIORITY APPLN. INFO.:	US 2000-182360P	P 20000214
	US 2000-191261P	P 20000322
	US 2001-784810	A3 20010214
	WO 2001-US4789	W 20010214

AB Amino acid and encoding cDNA sequences of two isoforms of **human sphingosine kinase** are disclosed. Amino acid and cDNA sequences of sphingosine kinases of rat and mouse are also provided. Also disclosed are antibodies that immunospecifically-bind to the sphingosine kinases, as well as derivs., variants, mutants, or fragments of the aforementioned polypeptide, polynucleotide, or antibody. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel human nucleic acids and proteins.

L6 ANSWER 37 OF 44 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER:	2002:6948 SCISEARCH
THE GENUINE ARTICLE:	502RX
TITLE:	Depolarisation induces rapid and transient formation of intracellular sphingosine-1-phosphate
AUTHOR:	Alemany R; Kleuser B; Ruwisch L; Danneberg K; Lass H; Hashemi R; Spiegel S; Jakobs K H; Heringdorf D M Z (Reprint)
CORPORATE SOURCE:	Univ Essen Gesamthsch Klinikum, Inst Pharmakol, Hufelandstr 55, D-45122 Essen, Germany (Reprint); Univ Essen Gesamthsch Klinikum, Inst Pharmakol, D-45122 Essen, Germany; Free Univ Berlin, Inst Pharm, D-14195 Berlin, Germany; Georgetown Univ, Med Ctr, Dept Biochem & Mol Biol, Washington, DC 20007 USA
COUNTRY OF AUTHOR:	Germany; USA
SOURCE:	FEBS LETTERS, (7 DEC 2001) Vol. 509, No. 2, pp. 239-244. ISSN: 0014-5793.
PUBLISHER:	ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE:	Article; Journal
LANGUAGE:	English
REFERENCE COUNT:	30
ENTRY DATE:	Entered STN: 5 Jan 2002 Last Updated on STN: 5 Jan 2002

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Formation of sphingosine-1-phosphate (SPP) by sphingosine kinase serves as a signalling pathway for various membrane receptors. Here, we show

that membrane depolarisation is another mechanism by which this pathway can be activated. Formation of [H-3]SPP as well as levels of endogenous SPP were rapidly and transiently increased in PC12 pheochromocytoma cells depolarised with high KCI. Time course and maximum were similar to those induced by bradykinin. Depolarisation-induced SPP production was also observed in RINm5F insulinoma cells, dependent on extracellular Ca2+ and fully suppressed by verapamil, thus apparently caused by Ca2+ influx via voltage-gated Ca2+ channels. Studies with sphingosine kinase inhibitors and overexpression of sphingosine kinase revealed a partial contribution of this pathway to depolarisation-induced noradrenaline release and Ca2+ increase. (C) 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

L6 ANSWER 38 OF 44 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:6934 SCISEARCH
THE GENUINE ARTICLE: 502RX
TITLE: A point mutant of **human sphingosine kinase** 1 with increased catalytic activity
AUTHOR: Pitson S M (Reprint); Moretti P A B; Zebol J R; Vadas M A; D'Andrea R J; Wattenberg B W
CORPORATE SOURCE: Hanson Ctr Canc Res, Div Human Immunol, Inst Med & Vet Sci, Frome Rd, Adelaide, SA 5000, Australia (Reprint); Hanson Ctr Canc Res, Div Human Immunol, Inst Med & Vet Sci, Adelaide, SA 5000, Australia; Univ Adelaide, Dept Med, Adelaide, SA 5000, Australia
COUNTRY OF AUTHOR: Australia
SOURCE: FEBS LETTERS, (7 DEC 2001) Vol. 509, No. 2, pp. 169-173. ISSN: 0014-5793.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 32
ENTRY DATE: Entered STN: 5 Jan 2002
Last Updated on STN: 5 Jan 2002

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Sphingosine kinase (SK) catalyses the formation of sphingosine I-phosphate, a lipid second messenger that has been implicated in mediating such fundamental biological processes as cell growth and survival. Very little is currently known regarding the structure or mechanisms of catalysis and activation of SK. Here ive have tested the functional importance of Gly(113), a highly conserved residue of **human sphingosine kinase** 1 (hSK), by site-directed mutagenesis. Surprisingly, a Gly(113) --> Ala substitution generated a mutant that had 1.7-fold greater catalytic activity than wild-type hSK (hSK(WT)). Our data suggests that the Gly(113) --> Ala mutation increases catalytic efficiency of hSK, probably by inducing a conformational change that increases the efficiency of phosphoryl transfer. Interestingly, hSK(G113A) activity could be stimulated in HEK293T cells by cell agonists to a comparable extent to hSK(WT). (C) 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

L6 ANSWER 39 OF 44 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 8

ACCESSION NUMBER: 2001-03254 BIOTECHDS
TITLE: Novel sphingosine-kinase protein and nucleic acid molecules for diagnosis, prophylaxis and treatment of rheumatoid arthritis, asthma, atherosclerosis, inflammation, meningitis, multiple sclerosis and septic shock;
involving vector plasmid pGEM4Z-mediated gene transfer for **expression** in Escherichia coli
AUTHOR: Pitson S M; Wattenberg B W; D'Andrea R J; Gamble J R; Vadas M

A
PATENT ASSIGNEE: Johnson+Johnson
LOCATION: Everleigh, New South Wales, Australia.
PATENT INFO: WO 2000070028 23 Nov 2000
APPLICATION INFO: WO 2000-AU457 12 May 2000
PRIORITY INFO: AU 1999-1504 8 Jul 1999; AU 1999-339 13 May 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-016227 [02]

AB An isolated sphingosine-kinase protein (I) or its derivative, analog, chemical equivalent or mimetic, is new. Also claimed are: an isolated nucleic acid molecule (II) or its derivative or analog comprising a nucleotide sequence encoding or complementary to a sequence encoding (I); an agent for use in modulating sphingosine-kinase activity or **expression**; a pharmaceutical composition (I) or the agent; an isolated antibody directed to (I) or (II); and diagnosing or monitoring a mammalian disease condition by screening for (I) in a biological sample isolated from the mammal. (I), (II) and the agent are useful for modulating **expression**, functional activity or cellular functional activity of sphingosine-kinase in a subject and also treating a mammal by modulating the activity of sphingosine-kinase. Diseases treated by regulating sphingosine-kinase cellular activity include rheumatoid arthritis, asthma, atherosclerosis, inflammation, meningitis, multiple sclerosis and septic shock. **Recombinant human sphingosine-kinase** was **expressed** by transforming the vector plasmid pGEM4Z into Escherichia coli BL21. (100pp)

L6 ANSWER 40 OF 44 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 9
ACCESSION NUMBER: 2000-14580 BIOTECHDS
TITLE: New **human sphingosine-kinase**-A, -B and -C polynucleotides and polypeptides useful in e.g. chromosome and gene mapping, and detecting inflammation or disease associated with abnormal levels of sphingosine-kinase **expression**;
vector-mediated gene transfer, **expression** in host cell, **recombinant** protein production, agonist, antagonist, antisense and DNA probe for disease therapy, diagnosis and gene therapy
AUTHOR: Munroe D; Gupta A; Falzone G R
PATENT ASSIGNEE: NPS-Allelix
LOCATION: Mississauga, Ontario, Canada.
PATENT INFO: WO 2000052173 8 Sep 2000
APPLICATION INFO: WO 2000-CA223 2 Mar 2000
PRIORITY INFO: US 990122516 2 Mar 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-572185 [53]

AB An isolated DNA (I) encoding **human sphingosine-kinase** (hSK)-A, -B and -C or their variants, is claimed. Also claimed are: an isolated DNA sequence complementary to (I); a composition with (I) and an excipient; a vector with (I); a host cell with the above vector; making a purified protein with the protein sequence for hSK by culturing the host cell and recovering the protein; a purified protein produced by the above method; and screening a compound for its antagonistic or agonistic properties against hSK activity by contacting the host cell with the compound and measuring the inhibition or activation of hSK activity. The hSK DNAs may be used as hybridization DNA probes, in the construction of oligomers for polymerase chain reaction, for chromosome gene mapping, in the **recombinant** production of hSK-A, -B and -C, and in the generation of antisense DNA or RNA. The DNA sequence for hSK can be used to detect inflammation or disease associated with abnormal levels of SK **expression**, or to

detect differences in gene sequence between normal and carrier or affected individuals. (81pp)

L6 ANSWER 41 OF 44 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 2000387082 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10751414
TITLE: Molecular **cloning** and functional characterization of a novel mammalian sphingosine kinase type 2 isoform.
AUTHOR: Liu H; Sugiura M; Nava V E; Edsall L C; Kono K; Poulton S; Milstien S; Kohama T; Spiegel S
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, D. C. 20007, USA.
CONTRACT NUMBER: GM43880 (NIGMS)
SOURCE: Journal of biological chemistry, (2000 Jun 30) 275 (26) 19513-20.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF245447; GENBANK-AF245448
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000818
Last Updated on STN: 20000818
Entered Medline: 20000810

AB Sphingosine-1-phosphate (SPP) has diverse biological functions acting inside cells as a second messenger to regulate proliferation and survival, and extracellularly, as a ligand for G protein-coupled receptors of the endothelial differentiation gene-1 subfamily. Based on sequence homology to murine and **human sphingosine kinase**-1 (SPHK1), which we recently **cloned** (Kohama, T., Oliver, A., Edsall, L. , Nagiec, M. M., Dickson, R., and Spiegel, S. (1998) J. Biol. Chemical 273, 23722-23728), we have now **cloned** a second type of mouse and **human sphingosine kinase** (mSPHK2 and hSPHK2). mSPHK2 and hSPHK2 encode proteins of 617 and 618 amino acids, respectively, both much larger than SPHK1, and though diverging considerably, both contain the conserved domains found in all SPHK1s. Northern blot analysis revealed that SPHK2 mRNA **expression** had a strikingly different tissue distribution from that of SPHK1 and appeared later in embryonic development. **Expression** of SPHK2 in HEK 293 cells resulted in elevated SPP levels. d-erythro-dihydrosphingosine was a better substrate than d-erythro-sphingosine for SPHK2. Surprisingly, d, l-threo-dihydrosphingosine was also phosphorylated by SPHK2. In contrast to the inhibitory effects on SPHK1, high salt concentrations markedly stimulated SPHK2. Triton X-100 inhibited SPHK2 and stimulated SPHK1, whereas phosphatidylserine stimulated both type 1 and type 2 SPHK. Thus, SPHK2 is another member of a growing class of sphingolipid kinases that may have novel functions.

L6 ANSWER 42 OF 44 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 2001097784 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10947957
TITLE: **Human sphingosine kinase**: purification, molecular **cloning** and characterization of the native and **recombinant** enzymes.
AUTHOR: Pitson S M; D'andrea R J; Vandeleur L; Moretti P A; Xia P; Gamble J R; Vadas M A; Wattenberg B W
CORPORATE SOURCE: Hanson Centre for Cancer Research, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide 5000, SA, Australia.
SOURCE: Biochemical journal, (2000 Sep 1) 350 Pt 2 429-41.
Journal code: 2984726R. ISSN: 0264-6021.

PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF200328
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010201

AB Sphingosine 1-phosphate (S1P) is a novel lipid messenger that has important roles in a wide variety of mammalian cellular processes including growth, differentiation and death. Basal levels of S1P in mammalian cells are generally low, but can increase rapidly and transiently when cells are exposed to mitogenic agents and other stimuli. This increase is largely due to increased activity of sphingosine kinase (SK), the enzyme that catalyses its formation. In the current study we have purified, **cloned** and characterized the first human SK to obtain a better understanding of its biochemical activity and possible activation mechanisms. The enzyme was purified to homogeneity from human placenta using ammonium sulphate precipitation, anion-exchange chromatography, calmodulin-affinity chromatography and gel-filtration chromatography. This resulted in a purification of over 10(6)-fold from the original placenta extract. The enzyme was **cloned** and **expressed** in active form in both HEK-293T cells and Escherichia coli, and the **recombinant** E. coli-derived SK purified to homogeneity. To establish whether post-translational modifications lead to activation of human SK activity we characterized both the purified placental enzyme and the purified **recombinant** SK produced in E. coli, where such modifications would not occur. The premise for this study was that post-translational modifications are likely to cause conformational changes in the structure of SK, which may result in detectable changes in the physico-chemical or catalytic properties of the enzyme. Thus the enzymes were characterized with respect to substrate specificity and kinetics, inhibition kinetics and various other physico-chemical properties. In all cases, both the native and **recombinant** SKs displayed remarkably similar properties, indicating that post-translational modifications are not required for basal activity of human SK.

L6 ANSWER 43 OF 44 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 2000263733 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10802064
TITLE: Functional characterization of **human sphingosine kinase**-1.
AUTHOR: Nava V E; Lacana E; Poulton S; Liu H; Sugiura M; Kono K; Milstien S; Kohama T; Spiegel S
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, 353 Basic Science Building, 3900 Reservoir Road NW, Washington, DC 20007, USA.
CONTRACT NUMBER: GM43880 (NIGMS)
SOURCE: FEBS letters, (2000 May 4) 473 (1) 81-4.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF238083
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000616
Last Updated on STN: 20000616
Entered Medline: 20000605

AB Sphingosine kinase catalyzes the phosphorylation of sphingosine to form sphingosine 1-phosphate (SPP), a novel lipid mediator with both intra- and

extracellular functions. Based on sequence identity to murine sphingosine kinase (mSPHK1a), we **cloned** and characterized the first **human sphingosine kinase** (hSPHK1). The open reading frame of hSPHK1 encodes a 384 amino acid protein with 85% identity and 92% similarity to mSPHK1a at the amino acid level. Similar to mSPHK1a, when HEK293 cells were transfected with hSPHK1, there were marked increases in sphingosine kinase activity resulting in elevated SPP levels. hSPHK1 also specifically phosphorylated D-erythro-sphingosine and to a lesser extent sphinganine, but not other lipids, such as D,L-threo-dihydrosphingosine, N, N-dimethylsphingosine, diacylglycerol, ceramide, or phosphatidylinositol. Northern analysis revealed that hSPHK1 was widely **expressed** with highest levels in adult liver, kidney, heart and skeletal muscle. Thus, hSPHK1 belongs to a highly conserved unique lipid kinase family that regulates diverse biological functions.

L6 ANSWER 44 OF 44 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 2000323213 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10863092
TITLE: **Human sphingosine kinase**: molecular **cloning**, functional characterization and tissue distribution.
AUTHOR: Melendez A J; Carlos-Dias E; Gosink M; Allen J M; Takacs L
CORPORATE SOURCE: Department of Molecular and Cellular Biology, Institut de Recherche Jouveinal/Parke-Davis, Fresnes, France.. alirio.melendez@wl.com
SOURCE: Gene, (2000 Jun 13) 251 (1) 19-26. Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000810
Last Updated on STN: 20000810
Entered Medline: 20000727

AB Sphingosine-1-phosphate (SPP), the product of sphingosine kinase, is an important signaling molecule with intra- and extracellular functions. The cDNA for the mouse sphingosine kinase has recently been reported. In this paper we describe the **cloning**, **expression** and characterization of the **human sphingosine kinase** (huSPHK1). Sequence analysis comparison revealed that this kinase is evolutionarily very conserved, having a high degree of homology with the murine enzyme, and presenting several conserved regions with bacteria, yeast, plant, and mammalian proteins. **Expressed** huSPHK1 cDNA specifically phosphorylates D-erythro-sphingosine and, to a lesser extent, D, L-erythro-dihydrosphingosine, and not at all the 'threo' isoforms of dihydrosphingosine; hydroxy-ceramide or non-hydroxy-ceramide; diacylglycerol (DAG); phosphatidylinositol (PI); phosphatidylinositol-4-phosphate (PIP); or phosphatidylinositol-4, 5-bisphosphate (PIP(2)). huSPHK1 shows typical Michaelis-Menten kinetics (V(max)=56microM and K(m)=5microM). The kinase is inhibited by D,L-threo-dihydrosphingosine (K(i)=3microM), and by N, N-dimethyl-sphingosine (K(i)=5microM). Northern blots indicate highest **expression** in adult lung and spleen, followed by peripheral blood leukocyte, thymus and kidney, respectively. It is also **expressed** in brain and heart. In addition, database searches with the stSG2854 sequence indicate that huSPHK1 is also **expressed** in endothelial cells, retinal pigment epithelium, and senescent fibroblasts.

```
=> e pitson s m/au
E1            4     PITSON L C/AU
E2           22     PITSON S/AU
E3           95 --> PITSON S M/AU
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E4            1     PITSON SM/AU
E5           11     PITSON STUART/AU
E6           68     PITSON STUART M/AU
E7            1     PITSON STUART MAXWELL/AU
E8            3     PITSOPOULOS C N/AU
E9           14     PITSOS M A/AU
E10           3     PITSOS N/AU
E11           1     PITSOTSKII B I/AU
E12          25     PITSOULAKIS G/AU

=> s e3
L7           95 "PITSON S M"/AU

=> e wattenberg b w/au
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E2            1     WATTENBERG B J/AU
E3          118 --> WATTENBERG B W/AU
E4            1     WATTENBERG BILL/AU
E5            4     WATTENBERG BINKS/AU
E6           46     WATTENBERG BINKS W/AU
E7            3     WATTENBERG BRIAN/AU
E8            1     WATTENBERG BRIAN W/AU
E9            2     WATTENBERG BRIAN WOLFF/AU
E10          12     WATTENBERG C A/AU
E11           2     WATTENBERG CARL A/AU
E12           4     WATTENBERG D/AU

=> s e3
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E3          473 --> XIA P/AU
E4            1     XIA P A/AU
E5           23     XIA P C/AU
E6            1     XIA P D K/AU
E7           32     XIA P F/AU
E8            1     XIA P G/AU
E9           12     XIA P J/AU
E10           1     XIA P L/AU
E11           6     XIA P Q/AU
E12           1     XIA P U/AU

=> s e3
L9          473 "XIA P"/AU

=> e gamble j r/au
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E2            1     GAMBLE J Q/AU
E3          361 --> GAMBLE J R/AU
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E5            7     GAMBLE J R JR/AU
E6            2     GAMBLE J S/AU
E7           26     GAMBLE J T/AU
E8           45     GAMBLE J W/AU
E9            2     GAMBLE JACKIE/AU
E10           1     GAMBLE JACQUI/AU
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E12          16     GAMBLE JAMES G/AU

=> s e3
L10         361 "GAMBLE J R"/AU
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E1            1      VADAS LUCIEN/AU
E2          115      VADAS M/AU
E3         1013 --> VADAS M A/AU
E4            1      VADAS M A */AU
E5            2      VADAS MA/AU
E6           37      VADAS MATHEW/AU
E7          216      VADAS MATHEW A/AU
E8           16      VADAS MATHEW ALEXANDER/AU
E9           10      VADAS MATTHEW/AU
E10          13      VADAS MATTHEW A/AU
E11           5      VADAS O/AU
E12         424      VADAS P/AU

=> s e3-e10
L11        1308 ("VADAS M A"/AU OR "VADAS M A *"/AU OR "VADAS MA"/AU OR "VADAS
                MATHEW"/AU OR "VADAS MATHEW A"/AU OR "VADAS MATHEW ALEXANDER"/AU
                 OR "VADAS MATTHEW"/AU OR "VADAS MATTHEW A"/AU)

=> d his

     (FILE 'HOME' ENTERED AT 14:21:23 ON 21 DEC 2005)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:24:12 ON 21 DEC 2005
L1         23924 S SPHINGOSINE
L2          2257 S L1(A)KINASE?
L3           113 S HUMAN (A)L2
L4       7440422 S CLON? OR EXPRESS? OR RECOMBINANT
L5            88 S L3 AND L4
L6            44 DUP REM L5 (44 DUPLICATES REMOVED)
                 E PITSON S M/AU
L7            95 S E3
                 E WATTENBERG B W/AU
L8           118 S E3
                 E XIA P/AU
L9           473 S E3
                 E GAMBLE J R/AU
L10          361 S E3
                 E VADAS M A/AU
L11         1308 S E3-E10

=> s l7 or l8 or l9 or l10 or l11
L12        1963 L7 OR L8 OR L9 OR L10 OR L11

=> s l2 and l12
L13         101 L2 AND L12

=> s human and l13
L14          77 HUMAN AND L13

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PROCESSING COMPLETED FOR L14
L15          24 DUP REM L14 (53 DUPLICATES REMOVED)

=> d 1-24 ibib ab

L15   ANSWER 1 OF 24  BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
      DUPLICATE 1
ACCESSION NUMBER: 2005-05093  BIOTECHDS
TITLE:            Modulating growth of a cell, useful for treatment and
                  prophylaxis of cancer, comprises contacting the cell with an
                  agent to modulate functional activity of sphingosine
                  kinase;
```

method of cancer gene therapy involving modulation of expression of **sphingosine-kinase**

AUTHOR: VADAS M; GAMBLE J; **XIA P**; WANG L; SUKOCHEVA O
PATENT ASSIGNEE: MEDVET SCI PTY LTD
PATENT INFO: US 2005009732 13 Jan 2005
APPLICATION INFO: US 2004-780897 19 Feb 2004
PRIORITY INFO: AU 2003-900729 19 Feb 2003; AU 2003-900729 19 Feb 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-090412 [10]

AB DERWENT ABSTRACT:

NOVELTY - Modulating the growth of a cell comprises contacting the cell with an agent for a time and under conditions sufficient to modulate the functional activity of **sphingosine kinase**.

DETAILED DESCRIPTION - Modulating the growth of a cell comprises contacting the cell with an agent for a time and under conditions sufficient to modulate the functional activity of **sphingosine kinase**, where down-regulation of the functional activity of **sphingosine kinase** down-regulates the growth and up-regulation of the functional activity of the **sphingosine kinase** up-regulates the cell growth. The down-regulation is to an oncogenic ineffective level, while the up-regulation is to an oncogenic effective level. INDEPENDENT CLAIMS are included for the following: (1) method for treatment or prophylaxis of a condition characterized by aberrant, unwanted or otherwise inappropriate cell growth in a mammal by administering the agent as above; (2) a composition comprising the agent together with a carrier and/or diluents; and (3) a method of diagnosing a condition or a predisposition or resistance to a condition characterized by aberrant, unwanted or otherwise inappropriate cell growth in a mammal comprising screening a biological sample from the mammal for the presence of **sphingosine kinase** or a nucleic acid molecule encoding **sphingosine kinase**.

BIOTECHNOLOGY - Preferred Method: In modulating the growth of a cell, the growth is proliferation, particularly uncontrolled proliferation. The modulation of proliferation or functional activity can be down-regulation or up-regulation .The cell is a neoplastic cell, especially malignant and particularly from the colon, stomach, lung, brain, bone, esophagus, pancreas, breast, ovary or uterus, especially a breast cell. The malignant cell has become transfected due to upregulation of an oncogene (especially Ras) or has become transformed by **sphingosine kinase** overexpression oncogenic activity.
In the treatment or prophylaxis and diagnosing methods, the mammal is **human**.

ACTIVITY - Cytostatic. No biological data given.

MECHANISM OF ACTION - **Sphingosine kinase** modulator.

USE - The methods, compositions and kinases are useful for the treatment or prophylaxis of a condition characterized by aberrant, unwanted or otherwise inappropriate cell growth in a mammal (claimed), e.g. cancer of the colon, stomach, lung, brain, bone, esophagus, pancreas, breast, ovary or uterus.

ADMINISTRATION - Dosage is 0.1 microg-2000 mg. Administration is respiratory, intratracheal, nasopharyngeal, intravenous, intraperitoneal, subcutaneous, intracranial, intradermal, intramuscular, intraocular, intrathecal, intracerebral, nasal, infusion, oral, rectal, patch and implant.

ADVANTAGE - Modulation of **sphingosine kinase** allows a novel approach to treatment of cellular proliferation.

EXAMPLE - No relevant examples given.(35 pages)

L15 ANSWER 2 OF 24 EMBASE DUPLICATE 2
ACCESSION NUMBER: 2005120184 EMBASE
TITLE: **Sphingosine kinase** 1 (SK1) is recruited

to nascent phagosomes in **human** macrophages: Inhibition of SK1 translocation by Mycobacterium tuberculosis.

AUTHOR: Thompson C.R.; Iyer S.S.; Melrose N.; VanOosten R.; Johnson K.; **Pitson S.M.**; Obeid L.M.; Kusner D.J.

CORPORATE SOURCE: Dr. D.J. Kusner, Division of Infectious Diseases, Department of Internal Medicine, Univ. of Iowa Carver Coll. of Med., 2501 Crosspark Road, Coralville, IA 52241, United States. david-kusner@uiowa.edu

SOURCE: Journal of Immunology, (15 Mar 2005) Vol. 174, No. 6, pp. 3551-3561.
Refs: 54
ISSN: 0022-1767 CODEN: JOIMA3

COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20050331
Last Updated on STN: 20050331

AB Mycobacterium tuberculosis (M.tb) is a leading cause of global infectious mortality. The pathogenesis of tuberculosis involves inhibition of phagosome maturation, leading to survival of M.tb within **human** macrophages. A key determinant is M.tb-induced inhibition of macrophage **sphingosine kinase** (SK) activity, which normally induces Ca(2+) signaling and phagosome maturation. Our objective was to determine the spatial localization of SK during phagocytosis and its inhibition by M.tb. Stimulation of SK activity by killed M.tb, live Staphylococcus aureus, or latex beads was associated with translocation of cytosolic SK1 to the phagosome membrane. In contrast, SK1 did not associate with phagosomes containing live M.tb. To characterize the mechanism of phagosomal translocation, live cell confocal microscopy was used to compare the localization of wild-type SK1, catalytically inactive SK1 (G82D), and a phosphorylation-defective mutant that does not undergo plasma membrane translocation (SK1(S225A)). The magnitude and kinetics of translocation of SK1(G82D) and SK1(S225A) to latex bead phagosomes were indistinguishable from those of wild-type SK1, indicating that novel determinants regulate the association of SK1 with nascent phagosomes. These data are consistent with a model in which M.tb inhibits both the activation and phagosomal translocation of SK1 to block the localized Ca (2+) transients required for phagosome maturation. Copyright .COPYRGT.2005 by The American Association of Immunologists, Inc.

L15 ANSWER 3 OF 24 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2005170340 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15632208
TITLE: **Sphingosine kinase**-1 enhances endothelial cell survival through a PECAM-1-dependent activation of PI-3K/Akt and regulation of Bcl-2 family members.

AUTHOR: Limaye Vidya; Li Xiaochun; Hahn Chris; Xia Pu; Berndt Michael C; **Vadas Mathew A**; Gamble Jennifer R

CORPORATE SOURCE: Hanson Institute, Institute of Medical and Veterinary Science, Adelaide, SA, Australia.

SOURCE: Blood, (2005 Apr 15) 105 (8) 3169-77. Electronic Publication: 2005-01-04.
Journal code: 7603509. ISSN: 0006-4971.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200505
ENTRY DATE: Entered STN: 20050402

Last Updated on STN: 20050514
Entered Medline: 20050513

AB Sphingosine-1-phosphate (S1P), the bioactive product of **sphingosine kinase** (SK) activation, is a survival factor for endothelial cells. The mechanism of SK-mediated survival was investigated in endothelial cells with moderately raised intracellular SK activity. Overexpression of SK mediated survival primarily through the activation of the phosphatidyl inositol 3-kinase (PI-3K)/protein kinase B (Akt/PKB) pathway and an associated up-regulation of the antiapoptotic protein B cell lymphoma gene 2 (Bcl-2) and down-regulation of the proapoptotic protein bisindolylmaleimide (Bcl-2 interacting mediator of cell death; Bim). In addition there was an up-regulation and dephosphorylation of the junctional molecule platelet endothelial cell adhesion molecule-1 (PECAM-1), which was obligatory for activation of the PI-3K/Akt pathway, for SK-induced cell survival, and for the changes in the apoptosis-related proteins. Thus, raised intracellular SK activity induced a molecule involved in cell-cell interactions to augment cell survival through a PI-3K/Akt-dependent pathway. This is distinct from the activation of both PI-3K/Akt and mitogen-activated protein kinase (MAPK) pathways seen with exogenously added S1P. Cells overexpressing SK showed enhanced survival under conditions of serum deprivation and absence of attachment to extracellular matrix, suggesting a role for SK in the regulation of vascular phenomena that occur under conditions of stress, such as angiogenesis and survival in unattached states, as would be required for a circulating endothelial cell.

L15 ANSWER 4 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2005575909 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16179586
TITLE: Activation of the **sphingosine kinase** -signaling pathway by high glucose mediates the proinflammatory phenotype of endothelial cells.
AUTHOR: Wang Lijun; Xing Xiao-Ping; Holmes Andrew; Wadham Carol; Gamble Jennifer R; **Vadas Mathew A**; Xia Pu
CORPORATE SOURCE: Division of Human Immunology, Hanson Institute, Institute of Medical and Veterinary Science, Adelaide, Australia.
SOURCE: Circulation research, (2005 Oct 28) 97 (9) 891-9.
Electronic Publication: 2005-09-22.
Journal code: 0047103. ISSN: 1524-4571.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200512
ENTRY DATE: Entered STN: 20051029
Last Updated on STN: 20051216
Entered Medline: 20051207

AB Vascular endothelial cells are key targets for hyperglycemic damage that facilitates vascular inflammation and the vasculopathy associated with diabetes mellitus. However, the mechanisms underlying this damage remain undefined. We now demonstrate that hyperglycemia induces activation of **sphingosine kinase** (SphK), which represents a novel signaling pathway that mediates endothelial damage under ambient high glucose conditions. SphK activity was significantly increased in aorta and heart of streptozotocin-induced diabetic rats. Interestingly, this increase in SphK activity was prevented by insulin treatment, which achieved euglycemia in the diabetic animals. Hyperglycemia-induced increase in SphK activity was also evident in endothelial cells that received long-term exposure to high glucose (22 mmol/L). Studies using a small interfering RNA strategy demonstrated that endogenous SphK1, but not SphK2, is the major isoenzyme that was activated by high glucose. In addition, an increase in SphK1 phosphorylation was detected in a protein kinase C- and extracellular signal-regulated kinase 1/2-dependent manner, which accounts for the high glucose-induced increases in SphK activity.

Importantly, inhibition of SphK1 by either a chemical inhibitor (N',N'-dimethylsphingosine) or expression of a dominant-negative mutant of SphK1 (SphK(G82D)), or SphK1-specific small interfering RNA, strongly protected endothelial cells against high glucose-induced damage, as characterized by an attenuation in the expression of proinflammatory adhesion molecules, adhesion of leukocytes to endothelial cells, and nuclear factor kappaB activation. Thus, interventions that target the SphK-signaling pathway may have the potential to prevent vascular lesions under hyperglycemic conditions.

L15 ANSWER 5 OF 24 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2005119964 EMBASE
TITLE: Enhancement of intracellular sphingosine-1-phosphate production by inositol 1,4,5-trisphosphate-evoked calcium mobilisation in HEK-293 cells: Endogenous sphingosine-1-phosphate as a modulator of the calcium response.
AUTHOR: Blom T.; Slotte J.P.; **Pitson S.M.**; Tornquist K.
CORPORATE SOURCE: K. Tornquist, Department of Biology, Abo Akademi University, BioCity, Artillerigatan 6, 20520 Turku, Finland. ktornqvi@abo.fi
SOURCE: Cellular Signalling, (2005) Vol. 17, No. 7, pp. 827-836. Refs: 42
ISSN: 0898-6568 CODEN: CESIEY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20050407
Last Updated on STN: 20050407

AB Sphingosine-1-phosphate (S1P) regulates many cellular functions, such as migration, differentiation and growth. The effects of S1P are thought to be primarily mediated by G-protein coupled receptors, but an intracellular function as a calcium releasing second messenger has also been proposed. Here we show that in HEK-293 cells, exogenous S1P mobilises sequestered calcium by a mechanism primarily dependent on the phospholipase C (PLC)/inositol 1,4,5-trisphosphate (IP(3)) pathway, and secondarily on the subsequent synthesis of intracellular S1P. Stimulating HEK-293 cells exogenously with S1P increased the production of both inositol phosphates and intracellular S1P. The calcium response was inhibited in cells treated with 2-APB, caffeine or U73122, showing that the PLC/IP(3) pathway for calcium release is activated in response to exogenous S1P. The calcium response was partially inhibited in cells treated with the **sphingosine kinase** inhibitor DMS and in cells expressing a catalytically inactive **sphingosine kinase**, showing that endogenously produced S1P is also involved. Importantly, 2-APB and U73122 inhibited the S1P-evoked production of intracellular S1P. S1P is therefore not likely a major calcium releasing second messenger in HEK-293 cells, but rather a secondary regulator of calcium mobilisation. .COPYRGT. 2004 Elsevier Inc. All rights reserved.

L15 ANSWER 6 OF 24 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 2005004399 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15623571
TITLE: Phosphorylation-dependent translocation of **sphingosine kinase** to the plasma membrane drives its oncogenic signalling.
AUTHOR: Pitson Stuart M; Xia Pu; Leclercq Tamara M; Moretti Paul A B; Zebol Julia R; Lynn Helen E; Wattenberg Binks W; **Vadas Mathew A**
CORPORATE SOURCE: Hanson Institute and Division of Human Immunology,

Institute of Medical and Veterinary Science, Adelaide SA 5000, Australia.. stuart.pitson@imvs.sa.gov.au
SOURCE: Journal of experimental medicine, (2005 Jan 3) 201 (1) 49-54. Electronic Publication: 2004-12-28. Journal code: 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200503
ENTRY DATE: Entered STN: 20050105
Last Updated on STN: 20050329
Entered Medline: 20050328

AB **Sphingosine kinase** (SK) 1 catalyzes the formation of the bioactive lipid sphingosine 1-phosphate, and has been implicated in several biological processes in mammalian cells, including enhanced proliferation, inhibition of apoptosis, and oncogenesis. **Human** SK (hSK) 1 possesses high instrinsic catalytic activity which can be further increased by a diverse array of cellular agonists. We have shown previously that this activation occurs as a direct consequence of extracellular signal-regulated kinase 1/2-mediated phosphorylation at Ser225, which not only increases catalytic activity, but is also necessary for agonist-induced translocation of hSK1 to the plasma membrane. In this study, we report that the oncogenic effects of overexpressed hSK1 are blocked by mutation of the phosphorylation site despite the phosphorylation-deficient form of the enzyme retaining full instrinsic catalytic activity. This indicates that oncogenic signaling by hSK1 relies on a phosphorylation-dependent function beyond increasing enzyme activity. We demonstrate, through constitutive localization of the phosphorylation-deficient form of hSK1 to the plasma membrane, that hSK1 translocation is the key effect of phosphorylation in oncogenic signaling by this enzyme. Thus, phosphorylation of hSK1 is essential for oncogenic signaling, and is brought about through phosphorylation-induced translocation of hSK1 to the plasma membrane, rather than from enhanced catalytic activity of this enzyme.

L15 ANSWER 7 OF 24 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-14417 BIOTECHDS
TITLE: Modulating mammalian endothelial cell functional characteristics such as viability, proliferation and differentiation, useful for treating tumor, rheumatoid arthritis, involves modulating functional level of **sphingosine kinase**;
useful for preparation of a medicament for gene therapy
AUTHOR: **GAMBLE J R**; VADAS M; PITSON S; **XIA P**; LIMAYE V
PATENT ASSIGNEE: MEDVET SCI PTY LTD
PATENT INFO: WO 2004035786 29 Apr 2004
APPLICATION INFO: WO 2003-AU1356 14 Oct 2003
PRIORITY INFO: AU 2003-902047 30 Apr 2003; AU 2002-952032 14 Oct 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-365161 [34]

AB DERWENT ABSTRACT:
NOVELTY - Modulating (M1) one or more mammalian endothelial cell functional characteristics, involves modulating the functional level of **sphingosine kinase**, where inducing over-expression of the **sphingosine kinase** level modulates one or more of the functional characteristics of the endothelial cell.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) use of an agent capable of modulating the functional level of **sphingosine kinase** in the manufacture of a medicament for (M1); (2) use of **sphingosine kinase** or a nucleic acid encoding **sphingosine kinase** in the

manufacture of a medicament for (M1); and (3) a pharmaceutical composition comprising modulatory agent and one or more carriers and/or diluents when used in (M1).

WIDER DISCLOSURE - The following are disclosed: (1) generating endothelial cells by (M1); and (2) endothelial cells generated by (M1).

BIOTECHNOLOGY - Preferred Method: In (M1), the endothelial cell is a vascular endothelial cell. The endothelial cell functional characteristic is up-regulatable by **sphingosine kinase** over-expression and the characteristic is one or more of viability, proliferation, differentiation, cell surface molecule expression, cytokine responsiveness or enhanced proliferation or viability. The cell surface molecule is an adhesion molecule. The functional characteristic is up-regulated. The endothelial cell functional characteristic is up-regulatable by **sphingosine kinase** over-expression and the characteristic is the induction of a pro-inflammatory phenotype or angiogenic phenotype or maintenance of the CD34+ endothelial cell progenitor phenotype. The pro-inflammatory phenotype is down-regulated. The angiogenic phenotype is up-regulated or down-regulated. The CD34+ progenitor phenotype is maintained. The modulation is up-regulation of **sphingosine kinase** levels and the up-regulation is achieved by introducing into the endothelial cell a nucleic acid molecule encoding **sphingosine kinase** or its functional equivalent, derivative or homologue or the **sphingosine kinase** expression product or its functional derivative, homologue, analogue, equivalent or mimetic. The modulation is achieved by contacting the endothelial cell with a proteinaceous or non-proteinaceous molecule which modulates transcriptional and/or translational regulation of the **sphingosine kinase** gene. Modulation is up-regulation of **sphingosine kinase** levels and the up-regulation is achieved by contacting the endothelial cell with a proteinaceous or non-proteinaceous molecule, which functions as an agonist of the **sphingosine kinase** expression product. The modulation is down-regulation of **sphingosine kinase** levels and the down-regulation is achieved by contacting the endothelial cell with a proteinaceous or non-proteinaceous molecule which functions as an antagonist to the **sphingosine kinase** expression product. The molecule is a mutant **sphingosine kinase** which mutant is characterized by substitution of the glycine residue at position 82 to aspartate. The endothelial cell activity is modulated in vivo or in vitro. In the method of using an agent capable of modulating **sphingosine kinase** in the manufacture of medicament, the agent is a proteinaceous or non-proteinaceous molecule, which modulates transcriptional and/or translational regulation of the **sphingosine kinase** gene, functions as an agonist of **sphingosine kinase** activity or functions as an antagonist of **sphingosine kinase** activity.

ACTIVITY - Vulnerary; Antiarthritic; Antirheumatic; Cytostatic; Antiangiogenic. No biological data given.

MECHANISM OF ACTION - Protein Kinase Modulator; **Sphingosine Kinases** Modulator; Gene Therapy. Adenovirus carrying the **sphingosine kinase** (SK) gene were used to transfect vascular endothelial cells. Overexpression of SK was measured and found to be increased by 5.17-fold. Use of DAPI stain under basal conditions and under serum deprivation conditions showed that cells overexpressing SK were less likely to undergo apoptosis. Caspase-3 activity was also measured and found to be suppressed under higher SK levels.

USE - For modulating mammalian endothelial cell functions such as viability, proliferation, differentiation, cell surface molecule expression, cytokine responsiveness or enhanced proliferation or viability. (M1) is also useful for prophylaxis and/or treatment of a condition characterized by aberrant or otherwise unwanted endothelial cell functioning in a mammal. The medicament manufactured using agent capable of modulating the functional level of **sphingosine kinase** or a nucleic acid encoding **sphingosine**

kinase, is useful for treating a condition characterized by aberrant or otherwise unwanted endothelial cell functioning in a mammal. The condition is vascular engraftment, wound repair, tissue or organ transplantation or the repair of devascularised tissue and the modulated endothelial cell functional characteristic is one or more of enhanced endothelial cell proliferation, enhanced endothelial cell viability or maintenance of the CD34+ progenitor phenotype. The condition is an inflammatory condition and the modulated endothelial cell functional characteristic is down-regulation of one or more of an endothelial cell inflammatory of angiogenic phenotype. The condition is rheumatoid arthritis. The condition is characterized by unwanted angiogenesis and the modulated endothelial cell functional characteristic is down-regulation of an endothelial cell angiogenic phenotype. The condition is a tumor (all claimed).

ADMINISTRATION - Administration of the modulatory agent is by oral, intravenous, intramuscular, intraperitoneal, subcutaneous, intradermal, suppository routes or implanting (e.g., using slow release molecules) at 0.1-1 mg/kg body weight/day.

EXAMPLE - To determine the effect on endothelial cell function of over-expression of **sphingosine kinase** (SK), HUVEC (**human** vascular endothelial cells) were infected with either retrovirus containing SK or adenovirus containing SK, at 1 plate forming units (pfu)/cell. This level of adenovirus infection was chosen since it resulted in similar levels of SK activity as tumor necrosis factor (TNF) alpha-stimulation of endogenous SK in endothelial cells, and similar levels of SK activity as was achieved with retrovirus-mediated gene delivery. To determine whether over-expression of SK results in changes to the endogenous phenotype of endothelial cells, the adhesion molecule expression was investigated on these infected cells. Retrovirus-mediated over-expression of SK up-regulated basal VCAM-1 expression. Adenoviral-mediated over-expression of SK resulted in a similar increase in VCAM-1 expression. In contrast to VCAM-1, basal E selectin expression was not altered in cells over-expressing SK generated by retroviral or adenoviral-mediated transfection. As over-expression of SK induced basal levels of VCAM-1. To determine whether these cells exhibited an altered response to stimulation with TNFalpha-induced up-regulation of VCAM-1 expression. Interestingly, cells over-expressing SK also showed an enhanced E Selectin response following stimulation with TNFalpha even though basal E Selectin expression was not altered. Over-expression of dominant-negative SK (G82D) significantly inhibited the induction of VCAM-1 and E Selectin in response to TNFalpha compared with empty vector (EV). Significant levels of both adhesion molecules were induced in cells over-expressing SK. Retroviral and adenoviral delivery of SK generated similar phenotypes in endothelial cells, that of enhanced expression of adhesion molecules and altered response to TNFalpha. However the adenoviral system enabled large number of cells to be rapidly generated. To determine whether the alteration in adhesion molecule expression resulting from intracellular over-expression of SK had functional consequences, neutrophil adhesion to endothelial cells was measured. In the basal state, cells over-expressing SK showed significant neutrophil adhesion, in contrast to control cells which did not bind neutrophils. Stimulation of endothelial cells with a low dose of TNFalpha (0.04 ng/ml) resulted in minimal neutrophil adhesion in control cells, but significantly greater adhesion to cells over-expressing SK. Consistent with a role for SK in mediating PMN adhesion, endothelial cells over-expressing the dominant-negative SK, G82D, inhibited PMN adhesion in response to stimulation with TNF alpha. To determine whether SK over-expression also enhances the ability of endothelial cells to form tubes. Endothelial cells were plated onto the complex basement membrane matrix, Matrigel, Equivalent numbers of cells over-expressing SK and EV were seeded, cells over-expressing SK had already commenced realignment whereas the EV cells remained disorganized. By 30 minutes cells over-expressing SK showed greater evidence of tube alignment compared with EV cells. By one hour tube formation by cells over-expressing SK was

highly developed compared with EV cells. By 18 hours, a time where tube formation was complete, both cells over-expressing SK and EV cells showed a similar pattern of tube formation. These results suggest that over-expression of SK stimulates the rate of tube formation. (91 pages)

L15 ANSWER 8 OF 24 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2004342307 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15246004
TITLE: An assay for **sphingosine kinase** activity using biotinylated sphingosine and streptavidin-coated membranes.
AUTHOR: Roberts Jane L; Moretti Paul A B; Darrow Andrew L; Derian Claudia K; **Vadas Mathew A**; Pitson Stuart M
CORPORATE SOURCE: Hanson Institute, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, SA 5000, Australia.
SOURCE: Analytical biochemistry, (2004 Aug 1) 331 (1) 122-9. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200501
ENTRY DATE: Entered STN: 20040713
Last Updated on STN: 20050129
Entered Medline: 20050128

AB **Sphingosine kinase** catalyses the phosphorylation of sphingosine to generate sphingosine 1-phosphate, a lipid signaling molecule implicated in roles in a diverse range of mammalian cell processes through its action as both a ligand for G-protein-coupled cell-surface receptors and an apparent intracellular second messenger. This paper describes a rapid, sensitive, and reproducible assay for **sphingosine kinase** activity using biotinylated sphingosine (biotinyl-Sph) as a substrate and capturing the phosphorylated product with streptavidin-coated membranes. We have shown that both **human sphingosine kinase** 1 and 2 (hSK1 and hSK2) can efficiently phosphorylate biotinyl-Sph, with K(m) values similar to those of sphingosine. The assay utilizing this substrate has high sensitivity for hSK1 and hSK2, with detection limits in the low-femtomole range for both purified recombinant enzymes. Importantly, we have also demonstrated the capacity of this assay to measure endogenous **sphingosine kinase** activity in crude cell extracts and to follow changes in this activity following **sphingosine kinase** activation. Together, these results demonstrate the potential utility of this assay in both cell-based analysis of **sphingosine kinase** signaling pathways and high-throughput screens for agents affecting **sphingosine kinase** activity in vitro.

L15 ANSWER 9 OF 24 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:28863 BIOSIS
DOCUMENT NUMBER: PREV200400030039
TITLE: Screening method for an agent having an effect on a **sphingosine kinase** signaling pathway.
AUTHOR(S): Gamble, Jennifer [Inventor, Reprint Author]; **Vadas, Mathew** [Inventor]; Xia, Pu [Inventor]; Barter, Phillip [Inventor]; Rye, Kerry-Anne [Inventor]; Wattenberg, Brian [Inventor]; Pitson, Stuart [Inventor]
CORPORATE SOURCE: South Australia, Australia
ASSIGNEE: Medvet Science Pty. Ltd., Adelaide, Australia
PATENT INFORMATION: US 6649362 20031118
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Nov 18 2003) Vol. 1276, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file.

ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 31 Dec 2003
Last Updated on STN: 31 Dec 2003

AB A screening method for identifying a therapeutic candidate for a coronary heart disease or an inflammatory condition is disclosed. The screening method tests for the presence or absence of an effect by a putative therapeutic agent on a component of a **sphingosine kinase** signaling pathway.

L15 ANSWER 10 OF 24 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:796515 HCAPLUS
DOCUMENT NUMBER: 139:303797
TITLE: Variants of mammalian **sphingosine kinase** with reduced catalytic activity and their use in controlling sphingosine-1-phosphate activated processes
INVENTOR(S): Pitson, Stuart M.; Xia, Pu; Moretti, Paul A.; Verwey, Julia R.; **Vadas, Mathew A.**; Wattenberg, Brian W.
PATENT ASSIGNEE(S): Medvet Science Pty. Ltd., Australia
SOURCE: PCT Int. Appl., 95 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003082322	A1	20031009	WO 2003-AU388	20030328
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2480661	AA	20031009	CA 2003-2480661	20030328
EP 1499343	A1	20050126	EP 2003-745226	20030328
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
JP 2005526809	T2	20050908	JP 2003-579859	20030328

PRIORITY APPLN. INFO.:			
AU 2002-1448	A	20020328	
AU 2002-1538	A	20020405	
AU 2002-1621	A	20020408	
AU 2002-951668	A	20020919	
AU 2003-900230	A	20030121	
WO 2003-AU388	W	20030328	

AB The present invention relates generally to a method of modulating cellular activity by modulating the activity of **sphingosine kinase** by modulating phosphorylation of the enzyme. Modulating phosphorylation of the enzyme modulates the activity of the enzyme and its ability to catalyze formation of the signaling mol. sphingosine-1-phosphate. The present invention still further extends to **sphingosine kinase** variants and to functional derivs., homologues or analogs, chemical equivalent and mimetics thereof exhibiting reduced and/or ablated capacity to undergo phosphorylation. The method and mols. of the present invention are useful, inter alia, in the treatment and/or prophylaxis of conditions characterized by aberrant,

unwanted or otherwise inappropriate cellular and/or **sphingosine kinase** functional activity. The present invention is further directed to methods for identifying and/or designing agents capable of modulating **sphingosine kinase** phosphorylation.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 11 OF 24 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2003510413 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14532121
TITLE: Activation of **sphingosine kinase** 1 by ERK1/2-mediated phosphorylation.
AUTHOR: Pitson Stuart M; Moretti Paul A B; Zebol Julia R; Lynn Helen E; Xia Pu; **Vadas Mathew A**; Wattenberg Binks W
CORPORATE SOURCE: Hanson Institute, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, SA 5000, Australia.. stuart.pitson@imvs.sa.gov.au
SOURCE: EMBO journal, (2003 Oct 15) 22 (20) 5491-500. Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200401
ENTRY DATE: Entered STN: 20031101
Last Updated on STN: 20040110
Entered Medline: 20040109

AB **Sphingosine kinase** 1 is an agonist-activated signalling enzyme that catalyses the formation of sphingosine 1-phosphate, a lipid second messenger that has been implicated in a number of agonist-driven cellular responses, including stimulation of cell proliferation, inhibition of apoptosis and expression of inflammatory molecules. Although agonist-induced stimulation of **sphingosine kinase** activity is critical in a number of signalling pathways, nothing has been known of the molecular mechanism of this activation. Here we show that this activation results directly from phosphorylation of **sphingosine kinase** 1 at Ser225, and present several lines of evidence to show compellingly that the activating kinase is ERK1/2 or a close relative. Furthermore, we show that phosphorylation of **sphingosine kinase** 1 at Ser225 results not only in an increase in enzyme activity, but is also necessary for translocation of the enzyme from the cytosol to the plasma membrane. Thus, these studies have elucidated the mechanism of agonist-mediated **sphingosine kinase** activation, and represent a key finding in understanding the regulation of **sphingosine kinase**/**sphingosine** 1-phosphate-controlled signalling pathways.

L15 ANSWER 12 OF 24 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2003458641 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12881510
TITLE: **Sphingosine kinase** transmits estrogen signaling in **human** breast cancer cells.
AUTHOR: Sukocheva Olga A; Wang Lijun; Albanese Nathaniel; Pitson Stuart M; **Vadas Mathew A**; Xia Pu
CORPORATE SOURCE: Signal Transduction Laboratory, Division of Human Immunology, Hanson Institute, Institute of Medical and Veterinary Science and University of Adelaide, Adelaide, South Australia 5000, Australia.
SOURCE: Molecular endocrinology (Baltimore, Md.), (2003 Oct) 17 (10) 2002-12. Electronic Publication: 2003-07-24. Journal code: 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200406
ENTRY DATE: Entered STN: 20031002
Last Updated on STN: 20040624
Entered Medline: 20040622

AB Current understanding of cytoplasmic signaling pathways that mediate estrogen action in **human** breast cancer is incomplete. Here we report that treatment with 17beta-estradiol (E2) activates a novel signaling pathway via activation of **sphingosine kinase** (SphK) in MCF-7 breast cancer cells. We found that E2 has dual actions to stimulate SphK activity, i.e. a rapid and transient activation mediated by putative membrane G protein-coupled estrogen receptors (ER) and a delayed but prolonged activation relying on the transcriptional activity of ER. The E2-induced SphK activity consequently activates downstream signal cascades including intracellular Ca2+ mobilization and Erk1/2 activation. Enforced expression of **human** SphK type 1 gene in MCF-7 cells resulted in increases in SphK activity and cell growth. Moreover, the E2-dependent mitogenesis were highly promoted by SphK overexpression as determined by colony growth in soft agar and solid focus formation. In contrast, expression of SphKG82D, a dominant-negative mutant SphK, profoundly inhibited the E2-mediated Ca2+ mobilization, Erk1/2 activity and neoplastic cell growth. Thus, our data suggest that SphK activation is an important cytoplasmic signaling to transduce estrogen-dependent mitogenic and carcinogenic action in **human** breast cancer cells.

L15 ANSWER 13 OF 24 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 8
ACCESSION NUMBER: 2003-10480 BIOTECHDS
TITLE: Modulating cytokine- or tumor necrosis factor-induced cellular activity, useful for treating or preventing a neoplastic condition, comprises modulating an intracellular **sphingosine kinase**-dependent signaling mechanism;
protein-induced cellular activity modulation and agonist and antagonist for use in disease therapy
AUTHOR: **XIA P**; WANG L; VADAS M; GAMBLE J; MORETTI P; PITSON S
PATENT ASSIGNEE: MEDVET SCI PTY LTD
PATENT INFO: WO 2002098458 12 Dec 2002
APPLICATION INFO: WO 2002-AU710 3 Jun 2002
PRIORITY INFO: AU 2001-9759 27 Dec 2001; AU 2001-5521 7 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-201282 [19]

AB DERWENT ABSTRACT:
NOVELTY - Modulating cytokine-induced or tumor necrosis factor (TNF)-induced cellular activity, comprises contacting the cell with an agent under conditions sufficient to modulate the interaction of **sphingosine kinase** with a TNF receptor-associated factor (TRAF), preferably TRAF2, where inducing the association up-regulates cellular activity, and inhibiting the association down-regulates cellular activity.

DETAILED DESCRIPTION - Modulating cytokine-induced or tumor necrosis factor (TNF)-induced cellular activity, comprises contacting the cell with an agent for a time and under conditions sufficient to modulate the interaction of **sphingosine kinase** with a TNF receptor-associated factor (TRAF), preferably TRAF2, where inducing or agonizing the association up-regulates the cellular activity, and inhibiting or antagonizing the association down-regulates the cellular activity. INDEPENDENT CLAIMS are included for the following: (1) treating and/or preventing a condition of aberrant, unwanted or inappropriate cytokine-induced or tumor necrosis factor (TNF)-induced cellular activity in a mammal; (2) detecting an agent capable of modulating the interaction

of TRAF with **sphingosine kinase** or its functional equivalent or derivative; (3) analyzing, designing and/or modifying an agent capable of interacting with the TRAF binding site of **sphingosine kinase** or its derivative and modulating at least one functional activity associated with the **sphingosine kinase**; (4) an agent described or identified in the methods cited above; and (5) a pharmaceutical condition comprising the modulatory agent described in the methods above, and one or more pharmaceutical carriers and/or diluents;

BIOTECHNOLOGY - Preferred Methods: The tumor necrosis factor (TNF)-induced cellular activity is the induction of anti-apoptotic characteristics, and modulation is down-regulation of the interaction of **sphingosine kinase** with TNF receptor-associated factor (TRAF). The TNF-induced cellular activity is the induction of pro-inflammatory, and the induction is down-regulation of the interaction of **sphingosine kinase** with TRAF. The agent binds, links or associates with the C-terminal region of **sphingosine kinase**, where the C-terminal region is the amino acid sequence of Pro-Pro-Glu Glu (I). The **sphingosine kinase** is preferably **human sphingosine kinase**, and the C-terminal region is the sequence of (I) at amino acid residue numbers 379-382 of a fully defined sequence of 384 amino acids (S1) given in the specification. Treating and/or preventing a condition of aberrant, unwanted or inappropriate cytokine-induced cellular activity in a mammal, comprises administering to the mammal an agent that modulates the interaction of **sphingosine kinase** with a TRAF, where inducing or agonizing the association up-regulates the cellular activity, and inhibiting or antagonizing the association down-regulates the cellular activity. Treating and/or preventing a condition of aberrant, unwanted or inappropriate TNF-induced cellular activity in a mammal, comprises administering to the mammal an agent that modulates the interaction of **sphingosine kinase** with a TRAF, preferably TRAF2, where inducing or agonizing the association up-regulates the cellular activity, and inhibiting or antagonizing the association down-regulates the cellular activity. The mammal is preferably **human** and the condition is a neoplastic condition. Detecting an agent capable of modulating the interaction of TRAF with **sphingosine kinase** or its functional equivalent or derivative, comprises contacting a cell or its extract containing the **sphingosine kinase** and TRAF or its functional equivalent or derivative with a putative agent, and detecting an altered expression phenotype associated with the interaction. TRAF is preferably TRAF2. The altered expression phenotype is an altered apoptosis profile or is modulation of the functional activity of **sphingosine kinase**. Analyzing, designing and/or modifying an agent capable of interacting with the TRAF binding site of **sphingosine kinase** or its derivative and modulating at least one functional activity associated with the **sphingosine kinase**, comprises contacting the **sphingosine kinase** or its derivative with a putative agent and assessing the degree of interactive complementarity of the agent with the binding site. The TRAF binding site is the C-terminal region of **sphingosine kinase**, which is a **human sphingosine kinase**, and the C-terminal region is the sequence of (I) at amino acid residue numbers 379-382 of the sequence of S1.

ACTIVITY - Cytostatic; Antiinflammatory; Antirheumatic; Antiarthritic. No biological data given.

MECHANISM OF ACTION - **Sphingosine Kinase** Inhibitor; **Sphingosine Kinase** Stimulator; TRAF Agonist 2; TRAF Antagonist 2.

USE - The agent is useful for manufacturing a medicament for treating a mammal with a condition of aberrant, unwanted or inappropriate cytokine-induced or tumor necrosis factor (TNF)-induced cellular activity (claimed). The methods are useful for modulating cytokine-induced or

TNF-induced cellular activity, or for treating or preventing a condition of aberrant, unwanted or inappropriate cytokine-induced or TNF-induced cellular activity in a mammal, such as neoplastic condition or inflammation (e.g. rheumatoid arthritis).

ADMINISTRATION - Dosage is about 0.1-1 mg/kg/day. Administration may be oral, intravenous, intraperitoneal, intramuscular, subcutaneous, intradermal, rectal, intratracheal, intracranial, intraocular, intrathecal, intracerebral, or intranasal.

EXAMPLE - **Human** embryonic kidney cell line 293T was transiently transfected with wild type TNF receptor-associated factor-2 (TRAF2), a dominant-negative TRAF2, or an empty vector. Over-expression of TRAF2 not only enhanced TNF-induced **sphingosine kinase** but also itself was capable of activating **sphingosine kinase** by two-fold compared with control transfectants. Immunoblotting assay showed equivalent expression levels of the transgenes in the presence or absence of TNF stimulation. (96 pages)

L15 ANSWER 14 OF 24 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
DUPLICATE 9
ACCESSION NUMBER: 2002-06038 BIOTECHDS
TITLE: Novel **sphingosine kinase** variants which exhibit reduced catalytic activity useful for modulating cellular functional activity and treating or preventing inflammatory, degenerative diseases and neoplastic conditions ; mutant **sphingosine-kinase** produced by site-directed mutagenesis useful for gene therapy and prophylaxis
AUTHOR: PITSON S; MORETTI P; ZEBOL J; **XIA P**; GAMBLE J; VADAS M; D'ANDREA R; WATTENBERG B
PATENT ASSIGNEE: MEDVET SCI PTY LTD
PATENT INFO: WO 2002000887 3 Jan 2002
APPLICATION INFO: WO 2000-AU730 28 Jun 2000
PRIORITY INFO: AU 2001-2749 29 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-130896 [17]
AB DERWENT ABSTRACT:

NOVELTY - A **sphingosine kinase** variant (I), comprising a mutation in a **sphingosine kinase** binding region defined by amino acids 16-153 or a ATP binding site region (or their functionally equivalent regions), where the variant exhibits ablated or reduced catalytic activity relative to wild-type SK, or a derivative, homolog, analog, chemical equivalent or mimetic of the SK variant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II), its derivative or equivalent, comprising a nucleotide sequence encoding or complementary to a sequence encoding (I); (2) detecting an agent capable of modulating the interaction of FOSK (friends of SK) with SK or its functional equivalent or derivative, by contacting a cell or its extract containing the SK or FOSK or its functional equivalent or derivative with a putative agent and detecting an altered expression phenotype associated with the interaction; (3) detecting an agent capable of binding or otherwise associating with the SK region defined by amino acid 16-153 (or its functional equivalent or derivative), by contacting a cell containing the amino acid region with a putative agent and detecting an altered expression phenotype associated with modulation of the function of SK; (4) analyzing, designing and/or modifying an agent capable of interacting with SK region defined by amino acid 16-153 or its derivative and modulating a functional activity associated with SK, by contacting SK or its derivative with a putative agent and assessing the degree of interactive complementarity of the agent with the binding site; (5) an

agent (II) identified by the method of (2), (3) or (4); and (6) a pharmaceutical composition comprising (I) or (II).

BIOTECHNOLOGY - Preparation: (I) is derived from natural or recombinant sources. Preferred Variant: SK is **human** SK and comprises a single or multiple amino acid substitution, addition and/or deletion. The SK binding region is defined by amino acids 70-90, preferably 79-84. The variant exhibits ablated catalytic activity, particularly a reduced capacity to phosphorylate sphingosine to sphingosine 1-phosphate.

ACTIVITY - Antiinflammatory; Antirheumatic; Antiarthritic; Cytostatic; Antiasthmatic; Antiatherosclerotic; Neuroprotective; Antibacterial; Immunosuppressive; Osteopathic. No biological data is given.

MECHANISM OF ACTION - Modulator of SK/FOSK interactivity; Inhibitor of wild-type SK activation; Regulator of cellular functional activity including chemokine, cytokine and inflammatory modulator production; gene therapy.

USE - (I) and (II) are useful for modulating cellular functional activity, down-regulating wild-type SK baseline activity and/or preventing wild-type SK activation. (I) and (II) are also useful for treatment and/or prophylaxis of a condition in a mammal, characterized by aberrant, unwanted or inappropriate cellular activity, and in the manufacture of a medicament for modulating cellular functional activity. (All claimed). (I) is useful in therapeutically or prophylactically treating inflammatory diseases (e.g. rheumatoid arthritis, inflammatory bowel disease), neoplastic conditions (e.g. solid cancer), asthma, atherosclerosis, meningitis, multiple sclerosis, septic shock, osteoarthritis, and other degenerative diseases.

ADMINISTRATION - Administered by oral, intravenous, intranasal, intraperitoneal, intramuscular, subcutaneous, intradermal or suppository route at a dose of 0.1-1 mg/kg/day.

EXAMPLE - The **sphingosine kinase** (SK-1) cDNA Pitson et al., 2000 was cloned into pALTER site directed mutagenesis vector. Single-stranded DNA was prepared and used as template for oligonucleotide directed mutagenesis. The mutagenic oligonucleotide (5'-CTGGAGACGATCTGATGCAC) was designed to generate the G82D mutant, substitution of the glycine at position 82 to aspartic acid. The mutagenic oligonucleotide (5'-GTCTGGAGATGCATTGATGCACG-3') was designed to generate the SK(G82A) mutant, substitution of the glycine at position 82 to alanine. The mutants were sequenced to verify incorporation of the desired modification and sub-cloned into pcDNA3. The expression construct was transfected by calcium phosphate precipitation into HEK293T cells. The G82DSK by itself had no SK activity and did not suppress endogenous baseline SK activity, however it totally suppressed the increases in SK activity seen after treatment of cells with activating agents such as tumor necrosis factor (TNF), interleukin-1 (IL-1) and PMA (phorbol-12-myristate-13-acetate). G82DSK inhibited SK stimulated by the oncogene Ras and suppressed in vitro and in vivo markers of oncogenesis. The inhibitor was specific as it didn't depress the activation of another enzyme protein kinase C or sphingomyelinase. **Human** SK(G82A) had catalytic activity much lower than the wild-type hSK. Analysis of the substrate kinetics of hSK(G82A) showed that this mutant had considerably lower affinity for ATP than the wild-type hSK, while the affinity for sphingosine remained unaffected. The kinetic data indicated that Gly82 was involved in ATP binding and this residue was a part of the ATP-binding site of HSK.(104 pages)

L15 ANSWER 15 OF 24 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 2002731982 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12393916
TITLE: The nucleotide-binding site of **human** **sphingosine kinase** 1.
AUTHOR: Pitson Stuart M; Moretti Paul A B; Zebol Julia R; Zareie Reza; Derian Claudia K; Darrow Andrew L; Qi Jenson;

D'Andrea Richard J; Bagley Christopher J; **Vadas Mathew A**; Wattenberg Binks W

CORPORATE SOURCE: Hanson Institute, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide SA 5000, Australia.. stuart.pitson@imvs.sa.gov.asu

SOURCE: Journal of biological chemistry, (2002 Dec 20) 277 (51) 49545-53. Electronic Publication: 2002-10-18. Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200302

ENTRY DATE: Entered STN: 20021227
Last Updated on STN: 20030214
Entered Medline: 20030212

AB **Sphingosine kinase** catalyzes the formation of sphingosine 1-phosphate, a lipid second messenger that has been implicated in a number of agonist-driven cellular responses including mitogenesis, anti-apoptosis, and expression of inflammatory molecules. Despite the importance of **sphingosine kinase**, very little is known regarding its structure or mechanism of catalysis. Moreover, **sphingosine kinase** does not contain recognizable catalytic or substrate-binding sites, based on sequence motifs found in other kinases. Here we have elucidated the nucleotide-binding site of **human sphingosine kinase** 1 (hSK1) through a combination of site-directed mutagenesis and affinity labeling with the ATP analogue, FSBA. We have shown that Gly(82) of hSK1 is involved in ATP binding since mutation of this residue to alanine resulted in an enzyme with an approximately 45-fold higher K(m)((ATP)). We have also shown that Lys(103) is important in catalysis since an alanine substitution of this residue ablates catalytic activity. Furthermore, we have shown that this residue is covalently modified by FSBA. Our data, combined with amino acid sequence comparison, suggest a motif of SGDGX(17-21)K is involved in nucleotide binding in the **sphingosine kinases**. This motif differs in primary sequence from all previously identified nucleotide-binding sites. It does, however, share some sequence and likely structural similarity with the highly conserved glycine-rich loop, which is known to be involved in anchoring and positioning the nucleotide in the catalytic site of many protein kinases.

L15 ANSWER 16 OF 24 MEDLINE on STN DUPLICATE 11

ACCESSION NUMBER: 2002139136 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11777919

TITLE: **Sphingosine kinase** interacts with TRAF2 and dissects tumor necrosis factor-alpha signaling.

AUTHOR: Xia Pu; Wang Lijun; Moretti Paul A B; Albanese Nathaniel; Chai Fugui; Pitson Stuart M; D'Andrea Richard J; Gamble Jennifer R; **Vadas Mathew A**

CORPORATE SOURCE: Division of Human Immunology, The Hanson Institute, Institute of Medical and Veterinary Science and University of Adelaide, Frome Road, Adelaide SA 5000, Australia.. pu.xia@imvs.sa.gov

SOURCE: Journal of biological chemistry, (2002 Mar 8) 277 (10) 7996-8003. Electronic Publication: 2002-01-02. Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200204

ENTRY DATE: Entered STN: 20020305
Last Updated on STN: 20030105
Entered Medline: 20020415

AB Tumor necrosis factor-alpha (TNF) receptor-associated factor 2 (TRAF2) is one of the major mediators of TNF receptor superfamily transducing TNF signaling to various functional targets, including activation of NF-kappa B, JNK, and antiapoptosis. We investigated how TRAF2 mediates differentially the distinct downstream signals. We now report a novel mechanism of TRAF2-mediated signal transduction revealed by an association of TRAF2 with **sphingosine kinase** (SphK), a lipid kinase that is responsible for the production of sphingosine 1-phosphate. We identified a TRAF2-binding motif of SphK that mediated the interaction between TRAF2 and SphK resulting in the activation of the enzyme, which in turn is required for TRAF2-mediated activation of NF-kappa B but not JNK. In addition, by using a kinase inactive dominant-negative SphK and a mutant SphK that lacks TRAF2-binding motif we show that the interaction of TRAF2 with SphK and subsequent activation of SphK are critical for prevention of apoptosis during TNF stimulation. These findings show a role for SphK in the signal transduction by TRAF2 specifically leading to activation of NF-kappa B and antiapoptosis.

L15 ANSWER 17 OF 24 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 2001700595 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11741582
TITLE: A point mutant of **human sphingosine kinase** 1 with increased catalytic activity.
AUTHOR: **Pitson S M**; Moretti P A; Zebol J R; **Vadas M A**; D'Andrea R J; **Wattenberg B W**
CORPORATE SOURCE: Hanson Centre for Cancer Research, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, SA 5000, Australia.. stuart.pitson@imvs.sa.gov.au
SOURCE: FEBS letters, (2001 Dec 7) 509 (2) 169-73. Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 20011220
Last Updated on STN: 20020125
Entered Medline: 20020117

AB **Sphingosine kinase** (SK) catalyses the formation of sphingosine 1-phosphate, a lipid second messenger that has been implicated in mediating such fundamental biological processes as cell growth and survival. Very little is currently known regarding the structure or mechanisms of catalysis and activation of SK. Here we have tested the functional importance of Gly(113), a highly conserved residue of **human sphingosine kinase** 1 (hSK), by site-directed mutagenesis. Surprisingly, a Gly(113)-->Ala substitution generated a mutant that had 1.7-fold greater catalytic activity than wild-type hSK (hSK(WT)). Our data suggests that the Gly(113)-->Ala mutation increases catalytic efficiency of hSK, probably by inducing a conformational change that increases the efficiency of phosphoryl transfer. Interestingly, hSK(G113A) activity could be stimulated in HEK293T cells by cell agonists to a comparable extent to hSK(WT).

L15 ANSWER 18 OF 24 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN DUPLICATE 13
ACCESSION NUMBER: 2001-03254 BIOTECHDS
TITLE: Novel **sphingosine-kinase** protein and nucleic acid molecules for diagnosis, prophylaxis and treatment of rheumatoid arthritis, asthma, atherosclerosis, inflammation, meningitis, multiple sclerosis and septic shock ;
involving vector plasmid pGEM4Z-mediated gene transfer for expression in Escherichia coli

AUTHOR: **Pitson S M; Wattenberg B W**; D'Andrea R J; **Gamble J R; Vadas M A**
PATENT ASSIGNEE: Johnson+Johnson
LOCATION: Everleigh, New South Wales, Australia.
PATENT INFO: WO 2000070028 23 Nov 2000
APPLICATION INFO: WO 2000-AU457 12 May 2000
PRIORITY INFO: AU 1999-1504 8 Jul 1999; AU 1999-339 13 May 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-016227 [02]

AB An isolated **sphingosine-kinase** protein (I) or its derivative, analog, chemical equivalent or mimetic, is new. Also claimed are: an isolated nucleic acid molecule (II) or its derivative or analog comprising a nucleotide sequence encoding or complementary to a sequence encoding (I); an agent for use in modulating **sphingosine-kinase** activity or expression; a pharmaceutical composition (I) or the agent; an isolated antibody directed to (I) or (II); and diagnosing or monitoring a mammalian disease condition by screening for (I) in a biological sample isolated from the mammal. (I), (II) and the agent are useful for modulating expression, functional activity or cellular functional activity of **sphingosine-kinase** in a subject and also treating a mammal by modulating the activity of **sphingosine-kinase**. Diseases treated by regulating **sphingosine-kinase** cellular activity include rheumatoid arthritis, asthma, atherosclerosis, inflammation, meningitis, multiple sclerosis and septic shock. Recombinant **human sphingosine-kinase** was expressed by transforming the vector plasmid pGEM4Z into Escherichia coli BL21. (100pp)

L15 ANSWER 19 OF 24 MEDLINE on STN DUPLICATE 14
ACCESSION NUMBER: 2001038285 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10944534
TITLE: Expression of a catalytically inactive **sphingosine kinase** mutant blocks agonist-induced **sphingosine kinase** activation. A dominant-negative **sphingosine kinase**.
AUTHOR: **Pitson S M**; Moretti P A; Zebol J R; **Xia P**; **Gamble J R**; **Vadas M A**; D'Andrea R J; **Wattenberg B W**
CORPORATE SOURCE: Hanson Centre for Cancer Research, Division of Human Immunology, Institute of Medical and Veterinary Science and the Department of Medicine, University of Adelaide, Frome Road, Adelaide, SA 5000, Australia.
SOURCE: Journal of biological chemistry, (2000 Oct 27) 275 (43) 33945-50.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001124

AB **Sphingosine kinase** (SK) catalyzes the formation of sphingosine 1-phosphate (S1P), a lipid messenger that plays an important role in a variety of mammalian cell processes, including inhibition of apoptosis and stimulation of cell proliferation. Basal levels of S1P in cells are generally low but can increase rapidly when cells are exposed to various agonists through rapid and transient activation of SK activity. To date, elucidation of the exact signaling pathways affected by these elevated S1P levels has relied on the use of SK inhibitors that are known to have direct effects on other enzymes in the cell. Furthermore, these inhibitors block basal SK activity, which is thought to have a

housekeeping function in the cell. To produce a specific inhibitor of SK activation we sought to generate a catalytically inactive, dominant-negative SK. This was accomplished by site-directed mutagenesis of Gly(82) to Asp of the **human** SK, a residue identified through sequence similarity to the putative catalytic domain of diacylglycerol kinase. This mutant had no detectable SK activity when expressed at high levels in HEK293T cells. Activation of endogenous SK activity by tumor necrosis factor-alpha (TNFalpha), interleukin-1beta, and phorbol esters in HEK293T cells was blocked by expression of this inactive **sphingosine kinase** (hSK(G82D)). Basal SK activity was unaffected by expression of hSK(G82D). Expression of hSK(G82D) had no effect on TNFalpha-induced activation of protein kinase C and sphingomyelinase activities. Thus, hSK(G82D) acts as a specific dominant-negative SK to block SK activation. This discovery provides a powerful tool for the elucidation of the exact signaling pathways affected by elevated S1P levels following SK activation. To this end we have employed the dominant-negative SK to demonstrate that TNFalpha activation of extracellular signal-regulated kinases 1 and 2 (ERK1,2) is dependent on SK activation.

L15 ANSWER 20 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2001115700 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11114522
TITLE: An oncogenic role of **sphingosine kinase**
.
AUTHOR: **Xia P**; **Gamble J R**; Wang L; **Pitson S M**; Moretti P A; **Wattenberg B W**; D'Andrea R J; **Vadas M A**
CORPORATE SOURCE: Division of Human Immunology, Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science and University of Adelaide, Frome Road, SA 5000,., Adelaide, Australia.. pu.xia@imvs.sa.gov.au
SOURCE: Current biology : CB, (2000 Nov 30) 10 (23) 1527-30. Journal code: 9107782. ISSN: 0960-9822.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010215

AB **Sphingosine kinase** (SphK) is a highly conserved lipid kinase that phosphorylates sphingosine to form sphingosine-1-phosphate (S1P). S1P/SphK has been implicated as a signalling pathway to regulate diverse cellular functions [1-3], including cell growth, proliferation and survival [4-8]. We report that cells overexpressing SphK have increased enzymatic activity and acquire the transformed phenotype, as determined by focus formation, colony growth in soft agar and the ability to form tumours in NOD/SCID mice. This is the first demonstration that a wild-type lipid kinase gene acts as an oncogene. Using a chemical inhibitor of SphK, or an SphK mutant that inhibits enzyme activation, we found that SphK activity is involved in oncogenic H-Ras-mediated transformation, suggesting a novel signalling pathway for Ras activation. The findings not only point to a new signalling pathway in transformation but also to the potential of SphK inhibitors in cancer therapy.

L15 ANSWER 21 OF 24 MEDLINE on STN DUPLICATE 15
ACCESSION NUMBER: 2001097784 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10947957
TITLE: **Human sphingosine kinase**: purification, molecular cloning and characterization of the native and recombinant enzymes.
AUTHOR: **Pitson S M**; D'andrea R J; Vandeleur L; Moretti P

A; **Xia P; Gamble J R; Vadas M A ; Wattenberg B W**

CORPORATE SOURCE: Hanson Centre for Cancer Research, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide 5000, SA, Australia.

SOURCE: Biochemical journal, (2000 Sep 1) 350 Pt 2 429-41. Journal code: 2984726R. ISSN: 0264-6021.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AF200328

ENTRY MONTH: 200102

ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010201

AB Sphingosine 1-phosphate (S1P) is a novel lipid messenger that has important roles in a wide variety of mammalian cellular processes including growth, differentiation and death. Basal levels of S1P in mammalian cells are generally low, but can increase rapidly and transiently when cells are exposed to mitogenic agents and other stimuli. This increase is largely due to increased activity of **sphingosine kinase** (SK), the enzyme that catalyses its formation. In the current study we have purified, cloned and characterized the first **human** SK to obtain a better understanding of its biochemical activity and possible activation mechanisms. The enzyme was purified to homogeneity from **human** placenta using ammonium sulphate precipitation, anion-exchange chromatography, calmodulin-affinity chromatography and gel-filtration chromatography. This resulted in a purification of over 10(6)-fold from the original placenta extract. The enzyme was cloned and expressed in active form in both HEK-293T cells and Escherichia coli, and the recombinant E. coli-derived SK purified to homogeneity. To establish whether post-translational modifications lead to activation of **human** SK activity we characterized both the purified placental enzyme and the purified recombinant SK produced in E. coli, where such modifications would not occur. The premise for this study was that post-translational modifications are likely to cause conformational changes in the structure of SK, which may result in detectable changes in the physico-chemical or catalytic properties of the enzyme. Thus the enzymes were characterized with respect to substrate specificity and kinetics, inhibition kinetics and various other physico-chemical properties. In all cases, both the native and recombinant SKs displayed remarkably similar properties, indicating that post-translational modifications are not required for basal activity of **human** SK.

L15 ANSWER 22 OF 24 MEDLINE on STN DUPLICATE 16

ACCESSION NUMBER: 2000036602 MEDLINE

DOCUMENT NUMBER: PubMed ID: 10567432

TITLE: Activation of **sphingosine kinase** by tumor necrosis factor-alpha inhibits apoptosis in **human** endothelial cells.

AUTHOR: **Xia P**; Wang L; **Gamble J R; Vadas M A**

CORPORATE SOURCE: Division of Human Immunology, The Hanson Centre for Cancer Research, Adelaide, South Australia 5000, Australia.

SOURCE: Journal of biological chemistry, (1999 Nov 26) 274 (48) 34499-505. Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199912

ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991229

AB **Human** umbilical vein endothelial cells (HUVEC), like most normal cells, are resistant to tumor necrosis factor-alpha (TNF)-induced apoptosis in spite of TNF activating sphingomyelinase and generating ceramide, a known inducer of apoptosis. Here we report that TNF activates another key enzyme, **sphingosine kinase** (SphK), in the sphingomyelin metabolic pathway resulting in production of sphingosine-1-phosphate (S1P) and that S1P is a potent antagonist of TNF-mediated apoptosis. The TNF-induced SphK activation is independent of sphingomyelinase and ceramidase activities, suggesting that TNF affects this enzyme directly other than through a mass effect on sphingomyelin degradation. In contrast to normal HUVEC, in a spontaneously transformed endothelial cell line (C11) TNF stimulation failed to activate SphK and induced apoptosis as characterized by morphological and biochemical criteria. Addition of exogenous S1P or increasing endogenous S1P by phorbol ester markedly protected C11 cell line from TNF-induced apoptosis. Conversely, N, N-dimethylsphingosine, an inhibitor of SphK, profoundly sensitized normal HUVEC to killing by TNF. Thus, we demonstrate that the activation of SphK by TNF is an important signaling for protection from the apoptotic effect of TNF in endothelial cells.

L15 ANSWER 23 OF 24 MEDLINE on STN DUPLICATE 17
ACCESSION NUMBER: 2000020293 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10551885
TITLE: High density lipoproteins (HDL) interrupt the **sphingosine kinase** signaling pathway. A possible mechanism for protection against atherosclerosis by HDL.
AUTHOR: **Xia P**; **Vadas M A**; Rye K A; Barter P J; **Gamble J R**
CORPORATE SOURCE: Division of Human Immunology, Hanson Centre for Cancer Research, Institute of Medical Science, University of Adelaide, Adelaide, South Australia 5000, Australia.
SOURCE: Journal of biological chemistry, (1999 Nov 12) 274 (46) 33143-7.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000114
Last Updated on STN: 20000114
Entered Medline: 20000103

AB The ability of high density lipoproteins (HDL) to inhibit cytokine-induced adhesion molecule expression has been demonstrated in their protective function against the development of atherosclerosis and associated coronary heart disease. A key event in atherogenesis is endothelial activation induced by a variety of stimuli such as tumor necrosis factor-alpha (TNF), resulting in the expression of various adhesion proteins. We have recently reported that sphingosine 1-phosphate, generated by **sphingosine kinase** activation, is a key molecule in mediating TNF-induced adhesion protein expression. We now show that HDL profoundly inhibit TNF-stimulated **sphingosine kinase** activity in endothelial cells resulting in a decrease in sphingosine 1-phosphate production and adhesion protein expression. HDL also reduced TNF-mediated activation of extracellular signal-regulated kinases and NF-kappaB signaling cascades. Furthermore, HDL enhanced the cellular levels of ceramide which in turn inhibits endothelial activation. Thus, the regulation of sphingolipid signaling in endothelial cells by HDL provides a novel insight into the mechanism of protection against atherosclerosis.

L15 ANSWER 24 OF 24 MEDLINE on STN DUPLICATE 18

ACCESSION NUMBER: 1999045661 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9826677
TITLE: Tumor necrosis factor-alpha induces adhesion molecule expression through the **sphingosine kinase** pathway.
AUTHOR: **Xia P**; **Gamble J R**; Rye K A; Wang L; Hii C S; Cockerill P; Khew-Goodall Y; Bert A G; Barter P J; **Vadas M A**
CORPORATE SOURCE: Division of Human Immunology, The Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science and University of Adelaide, Adelaide, SA 5000, Australia.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1998 Nov 24) 95 (24) 14196-201. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19981228

AB The signaling pathways that couple tumor necrosis factor-alpha (TNFalpha) receptors to functional, especially inflammatory, responses have remained elusive. We report here that TNFalpha induces endothelial cell activation, as measured by the expression of adhesion protein E-selectin and vascular adhesion molecule-1, through the **sphingosine kinase** (SKase) signaling pathway. Treatment of **human** umbilical vein endothelial cells with TNFalpha resulted in a rapid SKase activation and sphingosine 1-phosphate (S1P) generation. S1P, but not ceramide or sphingosine, was a potent dose-dependent stimulator of adhesion protein expression. S1P was able to mimic the effect of TNFalpha on endothelial cells leading to extracellular signal-regulated kinases and NF-kappaB activation, whereas ceramide or sphingosine was not. Furthermore, N, N-dimethylsphingosine, an inhibitor of SKase, profoundly inhibited TNFalpha-induced extracellular signal-regulated kinases and NF-kappaB activation and adhesion protein expression. Thus we demonstrate that the SKase pathway through the generation of S1P is critically involved in mediating TNFalpha-induced endothelial cell activation.

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(FILE 'HOME' ENTERED AT 14:21:23 ON 21 DEC 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:24:12 ON 21 DEC 2005

L1 23924 S SPHINGOSINE
L2 2257 S L1(A)KINASE?
L3 113 S HUMAN (A)L2
L4 7440422 S CLON? OR EXPRESS? OR RECOMBINANT
L5 88 S L3 AND L4
L6 44 DUP REM L5 (44 DUPLICATES REMOVED)
E PITSON S M/AU
L7 95 S E3
E WATTENBERG B W/AU
L8 118 S E3
E XIA P/AU
L9 473 S E3
E GAMBLE J R/AU
L10 361 S E3
E VADAS M A/AU

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L11         1308 S E3-E10
L12         1963 S L7 OR L8 OR L9 OR L10 OR L11
L13          101 S L2 AND L12
L14           77 S HUMAN AND L13
L15           24 DUP REM L14 (53 DUPLICATES REMOVED)
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	Issue Date	Pages	Document ID	Title
1	20051215	36	US 20050277612 A1	Methods, compositions and compound assays for inhibiting amyloid-beta protein production
2	20051201	35	US 20050266553 A1	Methods of regulating differentiation in stem cells
3	20051013	62	US 20050226862 A1	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders and for identifying agents therapeutic therefor
4	20051006	48	US 20050221346 A1	Sphingosine-1-phosphate lyase polypeptides, polynucleotides and modulating agents and methods of use therefor
5	20050922	216	US 20050208496 A1	Methods of testing for bronchial asthma or chronic obstructive pulmonary disease
6	20050825	16	US 20050187186 A1	Pharmaceutical compositions of safingol and methods of using the same
7	20050512	19	US 20050101674 A1	PPMP as a ceramide catabolism inhibitor for cancer treatment
8	20050512	39	US 20050100547 A1	Sphingosine kinase interacts with traf2 and modulates tumor necrosis factor-induced cellular activity

	Issue Date	Pages	Document ID	Title
9	20050407	24	US 20050074830 A1	Screening method for an agent having an affect on a sphingosine kinase signaling pathway
10	20050113	35	US 20050009732 A1	Method of treatment and agents useful for same
11	20041209	54	US 20040247603 A1	Compositions and methods for the treatment and prevention of cancer, angiogenesis, and inflammation
12	20041125	104	US 20040235071 A1	Methods and compositions for treating cancer using 15986, 2188, 20743, 9148, 9151, 9791, 44252, 14184, 42461, 8204, 7970, 25552, 21657, 26492, 2411, 15088, 1905, 28899, 63380, 33935, 10480, 12686, 25501, 17694, 15701, 53062, 49908, 21612, 38949, 6216, 46863, 9235, 2201, 6985, 9883, 12238, 18057, 21617, 39228, 49928, 54476, 62113, 64316, 12264, 32362, 58198, 2887, 3205, 8557, 9600, 9693, 44867, 53058, 55556, 57658, 2208, 10252, 10302, 14218, 33877, 10317, 10485, 25964, 14815, 1363, 1397, 14827, 21708, 3801, 64698, 2179 or 13249
13	20041028	34	US 20040214319 A1	Methods of regulating differentiation in stem cells

	Issue Date	Pages	Document ID	Title
14	20041014	42	US 20040203104 A1	Mammalian sphingosine kinase type 2 isoforms, cloning, expression and methods of use thereof
15	20040930	50	US 20040192580 A1	Regulation of human ceramide kinase
16	20040902	94	US 20040171037 A1	Amplified genes involved in cancer
17	20040708	58	US 20040132053 A1	Sphingosine kinase enzyme
18	20040701	101	US 20040126834 A1	Compositions and methods for the modulation of sphingolipid metabolism and/or signaling
19	20040624	31	US 20040120961 A1	Saposin C and receptors as targets for treatment of benign and malignant disorders
20	20040506	26	US 20040086487 A1	Induction of blood vessel formation through administration of polynucleotides encoding sphingosine kinases
21	20040325	82	US 20040058325 A1	Gene expression in biological conditions
22	20040318	287	US 20040053245 A1	Novel nucleic acids and polypeptides
23	20040219	30	US 20040034075 A1	Sphingosine kinase inhibitors
24	20040122	230	US 20040016025 A1	Rice promoters for regulation of plant expression

25	20040122	20	US 2004001463 5 A1	Sphingosine kinase and uses thereof

	Issue Date	Pages	Document ID	Title
26	20040108	345	US 20040005563 A1	Methods of diagnosis of ovarian cancer, compositions and methods of screening for modulators of ovarian cancer
27	20031127	81	US 20030219782 A1	Compositions and methods for the modulation of sphingolipid metabolism and/or signaling
28	20031009	40	US 20030190650 A1	Screening method
29	20030918	49	US 20030175939 A1	Sphingosine-1-phosphate lyase polypeptides, polynucleotides and modulating agents and methods of use therefor
30	20030911	55	US 20030170856 A1	Regulation of human map kinase phosphatase-like enzyme
31	20030911	41	US 20030170245 A1	Activation of matriptase and diagnostic and therapeutic methods based thereon
32	20030828	22	US 20030162206 A1	Ceramide kinase and DNA encoding it

	Issue Date	Pages	Document ID	Title
33	20030821	80	US 20030157082 A1	Methods and compositions for treating cancer using 140, 1470, 1686, 2089, 2427, 3702, 5891, 6428, 7181, 7660, 25641, 69583, 49863, 8897, 1682, 17667, 9235, 3703, 14171, 10359, 1660, 1450, 18894, 2088, 32427, 2160, 9252, 9389, 1642, 85269, 10297, 1584, 9525, 14124, 4469, 8990, 2100, 9288, 64698, 10480,20893, 33230,1586, 9943, 16334, 68862, 9011, 14031, 6178, 21225, 1420, 32236, 2099, 2150, 26583, 2784, 8941, 9811, 27444, 50566 or 66428 molecules
34	20030703	47	US 20030125533 A1	Regulation of human sphingosine kinase-like protein
35	20030522	61	US 20030096022 A1	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
36	20030206	60	US 20030027304 A1	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor

	Issue Date	Pages	Document ID	Title
46	20050419	64	US 6881546 B2	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
47	20050222	66	US 6858383 B2	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
48	20041214	22	US 6830916 B2	Sphingosine kinase, cloning, expression and methods of use
49	20041214	46	US 6830881 B2	Sphingosine-1-phosphate lyase polypeptides, polynucleotides and modulating agents and methods of use therefor
50	20041005	41	US 6800470 B2	Mammalian sphingosine kinase type 2 isoforms, cloning, expression and methods of use thereof
51	20040504	60	US 6730480 B1	Sphingosine kinase enzyme
52	20040420	19	US 6723525 B2	Methods and compositions for screening modulators of lipid kinases
53	20031118	23	US 6649362 B2	Screening method for an agent having an effect on a sphingosine kinase signaling pathway

	Issue Date	Pages	Document ID	Title
54	20030826	25	US 6610534 B2	Induction of blood vessel formation through administration of polynucleotides encoding sphingosine kinases
55	20030318	18	US 6534323 B1	Compositions and methods for early detection of heart disease
56	20030318	18	US 6534322 B1	Kits for early detection of heart disease
57	20021119	59	US 6482609 B1	Isolated human EDG-4 receptor and polynucletide encoding said receptor
58	20010403	19	US 6210976 B1	Methods for early detection of heart disease
59	19971014	8	US 5677189 A	Method for quantifying sphingosine and for diagnosing platelet activation

	Issue Date	Pages	Document ID	Title
1	20051215	36	US 20050277612 A1	Methods, compositions and compound assays for inhibiting amyloid-beta protein production
2	20051201	35	US 20050266553 A1	Methods of regulating differentiation in stem cells
3	20051013	62	US 20050226862 A1	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders and for identifying agents therapeutic therefor
4	20041209	54	US 20040247603 A1	Compositions and methods for the treatment and prevention of cancer, angiogenesis, and inflammation
5	20041028	34	US 20040214319 A1	Methods of regulating differentiation in stem cells
6	20040708	58	US 20040132053 A1	Sphingosine kinase enzyme
7	20030522	61	US 20030096022 A1	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor

	Issue Date	Pages	Document ID	Title
8	20030206	60	US 20030027304 A1	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
9	20030206	60	US 20030026799 A1	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
10	20050419	64	US 6881546 B2	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
11	20050222	66	US 6858383 B2	Compositions and methods for the treatment and prevention of cardiovascular diseases and disorders, and for identifying agents therapeutic therefor
12	20040504	60	US 6730480 B1	Sphingosine kinase enzyme

	L #	Hits	Search Text
1	L1	1	"6730480".pn.
2	L2	138	sphingosine adj kinase$2
3	L3	59	l2 same human
4	L4	796350	clon$3 or express$3 or recombinant
5	L5	42	l3 same l4
6	L6	254666	WATTENBERG XIA GAMBLE VADAS PITSON
7	L7	12	l3 same l6